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PHD THESIS

Assessing the patterns and causes of diversity in free-living unicellular eukaryotes (protists) using biogeographical, macroecological and landscape genetic approaches

Author:

Leonardo D. FERNÁNDEZ

Thesis committee:

Prof. Edward A. D. MITCHELL

Dr. Enrique LARA

Dr. Cristián E. HERNÁNDEZ

Dr. Margarita MARCHANT

IMPRIMATUR POUR THESE DE DOCTORAT

**La Faculté des sciences de l'Université de Neuchâtel
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**Monsieur Leonardo David
FERNANDEZ PARRA**

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sur le rapport des membres du jury composé comme suit:

- Prof. Edward Mitchell, co-directeur de thèse, Université de Neuchâtel, Suisse
- Dr Cristián E. Hernández U., co-directeur de thèse, Universidad de Concepción, Chili
- Dr Enrique Lara, Université de Neuchâtel, Suisse
- Dr Margarita Marchant, Universidad de Concepción, Chili

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Le Doyen, Prof. R. Bshary



Abstract

This PhD thesis tested the hypothesis that, at broad spatial scales, the patterns and underlying causes of diversity and population genetic structure of unicellular eukaryotes (protists) are similar to those observed for multicellular organisms (plants and animals).

To test this hypothesis, this thesis investigated (1) the pattern and causes of latitudinal diversity in soil testate amoebae (a polyphyletic group mainly represented by Arcellinida and Euglyphida) along southwestern South America, Chile (from 18 to 56 °S), Chile; (2) the patterns and causes of altitudinal diversity in several groups of soil protists (Bacillariophyta, Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida) along an elevation gradient of beech forests in the Swiss lowlands and Jura Mountains; and (3) the pattern and causes of population genetic structure in a freshwater eukaryotic microalga (Chlorophyta: Mamiellophyceae) in the Argentinean Patagonia.

Overall, the analyses revealed that at broad spatial scales, protists have patterns of latitudinal diversity, altitudinal diversity and population genetic structure similar to those of multicellular organisms. In addition, the evidence revealed that these patterns are originated and maintained by a combination of ecological, historical and evolutionary processes. Therefore, this thesis supported the hypothesis that the patterns and causes that produce and maintain the diversity of plants and animals at different ecological levels of organization could also be valid for microorganisms. The model organisms used in this thesis are also good representatives of the diversity of trophic, morphological, locomotive, physiological and probably reproductive strategies exhibited by protists. Consequently, the results of this thesis could be valid for a large number of eukaryotic microorganisms.

Key words: beta diversity, biogeography, community assembly, eukaryotic microorganism, landscape genetics, macroecology, niche-based processes, phylogenetic niche conservatism

Résumé

Cette thèse de doctorat a permis de tester l'hypothèse qu'à large échelle spatiale, les patrons observés et les causes qui influencent la diversité et la structure génétique des populations d'eucaryotes unicellulaires (protistes) sont similaires à ceux observées chez des organismes multicellulaires (plantes et animaux).

Afin de tester cette hypothèse, cette thèse s'est penchée sur (1) les patrons observés et les causes qui influencent la diversité des amibes à thèque (un groupe polyphylétique principalement représenté par les *Euglyphida* et *Arcellinida*) le long d'un gradient latitudinal à travers le Chili (de 18 à 56 °S); (2) les patrons observés et les causes qui influencent la diversité de différents groupes de protistes du sol (*Bacillariophyta*, *Cercomonadida*, *Ciliophora*, *Euglyphida* et *Kinetoplastida*) en fonction de l'altitude dans des forêts de hêtre dans le Plateau Suisse et le Jura; et (3) les patrons observés et les causes qui influencent la structure génétique des populations d'une micro-algue d'eau douce (*Chlorophyta*: *Mamiellophyceae*) dans la Patagonie Argentine.

Globalement, les analyses ont montré qu'à large échelle spatiale, les protistes ont des patrons de diversité latitudinale et altitudinale, ainsi qu'une structuration génétique semblable à ceux que l'on retrouve chez des organismes multicellulaires. De plus, les résultats montrent que ces patrons sont créés et maintenus par une combinaison de processus écologiques, historiques et évolutifs. Cette thèse a donc renforcé l'hypothèse qui considère que les patrons observés et les causes qui maintiennent la diversité des plantes et des animaux à différents niveaux écologiques d'organisation sont aussi effectifs chez les microorganismes. Les organismes modèles choisis dans cette thèse peuvent être considérés comme représentatifs de la diversité de stratégies trophiques, de morphologies, modes de locomotion ainsi que de la variété de physiologies et probablement aussi de modes de reproduction que l'on rencontre chez les protistes. Par conséquent, les résultats de cette thèse peuvent être considérés comme valides chez un grand nombre d'eucaryotes microscopiques.

Mots clés : diversité beta, biogéographie, assemblage de communautés, microorganisme eucaryote, génétique à l'échelle du paysage, macroécologie, processus basés sur la niche écologique, conservation phylogénétique de la niche

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Chapter 1: Introduction

Introduction

Currently, there is growing appreciation for the idea that the patterns of diversity and population genetic structure in multicellular organisms (e.g. plants and animals) result from a combination of ecological (e.g. abiotic factors, biotic interactions), historical (e.g. climatic stability) and evolutionary (spatial variation in diversification rates) processes, which together influence the spatial variation in speciation, extinction and dispersal rates (Wiens and Donoghue, 2004; Mittelbach et al., 2007; Barret et al., 2008). Although not equivalent, historical and evolutionary processes highlight the role that long-term dynamics play on the formation of biodiversity patterns of multicellular organisms. Accordingly, they are often jointly referred to as long-term processes (Mittelbach et al., 2007; Rivadeneira et al., 2011).

While the patterns and causes that originate and maintain multicellular diversity on Earth are becoming better understood, it is still not clear whether single-celled organisms (i.e. microorganisms) also exhibit patterns of diversity and population genetic structure; and if so, whether these patterns are produced and maintained by the same processes proposed for multicellular organisms. This represents a serious gap in knowledge given that microorganisms are the most diverse and abundant organisms on Earth (Whitman et al., 1998; López-García et al., 2001; Chao et al., 2006; de Vargas et al., 2015) and play pivotal roles in biogeochemical cycles (Adl and Gupta, 2006; Massana, 2011).

The patterns and causes of diversity and population genetic structure have been historically less explored in microorganisms simply because they are difficult to study. Except for a few cases (e.g. the bacterium *Thiomargarita namibiensis* with cells up to 0.75 mm in diameter; Schulz et al., 1999; or the foraminiferan *Heterostegina depressa* with a shell up to 3-5 mm in diameter; Hallock, 1985) they can only be observed under the microscope. Even if a microscope is used, there is little chance of detecting all microbial taxa present in a sample because many are in a dormant (cystic) stage waiting for optimal conditions to become active (Foissner, 1991, 1997; Weisse, 2008). Also, identifying and counting microorganisms is time-consuming and requires well trained taxonomists (Foissner and Hawksworth, 2009). These difficulties have limited the study of microbial diversity, resulting in much speculation and discussion about the patterns and processes that originate and maintain their diversity at different levels of organization. For instance, it has been hypothesized that microorganisms do not have biogeography and population

genetic structure because their tiny size might facilitate their passive dispersal from and to all regions of the planet via different vectors, such as wind, marine currents, migratory animals and humans (Finlay, 2002; Fenchel and Finlay, 2004).

Over the last decade, there has been much interest in explicitly testing this hypothesis. This trend has been stimulated by the increasing availability of exhaustive microbial species checklists from different regions of the planet and the development and application of DNA-based methods, such as Illumina. Exhaustive species checklists often are constructed from data gathered during several years and therefore provide a comprehensive record of the species present in a region (e.g. Dolan et al., 2006, Azovsky and Mazei, 2013, Ostojić et al., 2014; Fernández et al., 2015). Likewise, Illumina-based technologies facilitate the retrieval of hundreds of thousands of microbial barcodes per sample, allowing a comprehensive analysis of diversity and composition of natural microbial communities and populations (Lepère et al., 2013; Logares et al., 2014; de Vargas et al., 2015; Mahé et al., 2016; Schiaffino et al., 2016; Singer et al., 2016). Therefore, both exhaustive microbial species checklists and DNA-based methods nowadays allow a much more robust analysis of the patterns and causes of microbial diversity at broad-spatial scales.

Although some studies have found support for the hypothesis that microorganisms do not have biogeography (Finlay and Fenchel, 1999, 2004) or population genetic structure (Evans et al., 2005; Šlapeta et al., 2006; Casteleyn et al., 2010; Van Gremberghe et al., 2011), a growing body of evidence support the idea that most microorganisms do have predictable patterns of diversity that mirror those of multicellular organisms, including latitudinal gradients in diversity (Hewson and Fuhrman, 2004; Fuhrman et al., 2008; Lacap et al., 2011; Bates et al., 2013; Fernández et al., 2016; Lara et al., 2016; Schiaffino et al., 2016), altitudinal gradients in diversity (Bryant et al., 2008; Heger et al., 2015; Wang et al., 2016) and population genetic structure (Kim et al., 2004; Ryneerson and Armbrust, 2004; Nagai et al., 2007; Logares et al., 2009; Godhe and Härnström, 2010; Härnström et al., 2011; Sjöqvist et al., 2015).

While this recent research has contributed to better understand the spatial patterns of microbial diversity at different levels of ecological organization (communities, population), we do not know yet whether their findings are valid for all single-celled organisms in the planet. Most research has been focused on the Holarctic region, whereas other regions of the planet remain comparatively poorly explored (Fenchel and Finlay,

2003; Mitchell and Meisterfeld, 2005). Also, most research on microbial diversity has been conducted on prokaryotic or aquatic microbial taxa, while free-living soil eukaryotic microorganisms (i.e. soil protists) have been less-studied (Foissner, 1999; Chao et al., 2006; Bates et al., 2013; Fernández et al., 2016; Fournier et al., 2016; Lara et al., 2016). Presumably, this trend is driven by the smaller size of the global scientific community specialized in the study of soil protists. Finally, most research invokes ecological mechanisms to explain the occurrence of spatial patterns of microbial diversity at different levels of ecological organization (i.e. mostly assessing correlations between present-day environmental factors and diversity), neglecting the potential role played by long-term processes in shaping microbial diversity. Together, all these facts have prevented the construction of universal patterns and causes to explain microbial diversity at broad-spatial scales (Martiny et al., 2006). Accordingly, it is also not known to what extent microbial diversity conforms to the ecological principles proposed for plants and animals. Such gaps in knowledge defeat one of the major principles of ecology, namely “the construction of generalizations for all living entities” (Wilson et al., 2007).

From the above, it follows that it is urgent (1) to re-evaluate the spatial patterns of diversity and population genetic structure in protists, particularly in historically sub-sampled regions; (2) to investigate the ecological and long-term causes that underlie these patterns; and (3) to determine whether the patterns and causes that originate and maintain microbial diversity mirror those of multicellular organisms.

This PhD thesis tested the hypothesis that, at broad spatial scales, the patterns and causes of diversity and population genetic structure in protists are similar to those observed for multicellular organisms. To evaluate this hypothesis, this thesis adopted an interdisciplinary approach, including biogeographical, macroecological and landscape genetics methods.

This thesis consisted of four main studies. The first study (Chapter 2) aimed to give an updated and comprehensive checklist of soil testate amoeba species from Chile, a country stretching over an under-sampled region in terms of microbial diversity. This goal was achieved by bringing together more than 170 years of published and unpublished data on soil testate amoebae from this country. Another goal of this chapter was to use this information to conduct descriptive biogeography and propose possible causes underlying soil testate amoeba geographic distribution.

The second study (Chapter 3) aimed to explicitly test the ecological and long-term causes driving the latitudinal gradient of soil testate amoeba diversity in Chile. This goal was achieved using a combination of biogeographical and macroecological approaches and the species checklist generated in the previous chapter as input data.

The third study (Chapter 4) aimed to study the patterns and causes of diversity in several soil protist groups (i.e. Bacillariophyta, Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida) along an elevational gradient of beech forests in Switzerland. This goal was also achieved using a combination of biogeographical and macroecological approaches. However, this time, protist diversity data was obtained using a next-generation DNA sequencing approach.

Finally, the fourth study (Chapter 5) aimed to assess the pattern and causes of population genetic structure and differentiation in a eukaryotic freshwater microalga that inhabits lakes of the Argentinean Patagonia, another under-sampled region of the planet in terms of microbial diversity. This goal was achieved using landscape genetic approaches and a DNA barcoding approach to record the occurrence of this aquatic protist in each lake.

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Chapter 2: Checklist, diversity and distribution of testate amoebae in Chile



Checklist, diversity and distribution of testate amoebae in Chile

Leonardo D. Fernández^{a,b,c,*}, Enrique Lara^a, Edward A.D. Mitchell^{a,d}

^aLaboratory of Soil Biology, Institute of Biology, University of Neuchâtel, Rue Emile Argand 11, CH-2000, Neuchâtel, Switzerland

^bLaboratorio de Ecología Evolutiva y Filoinformática, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario s/n, Casilla 160-C, Concepción, Chile

^cCentro de Estudios en Biodiversidad (CEBCH), Magallanes 1979, Osorno, Chile

^dBotanical Garden of Neuchâtel, Chemin du Perthuis-du-Sault 58, CH-2000 Neuchâtel, Switzerland

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Abstract

Bringing together more than 170 years of data, this study represents the first attempt to construct a species checklist and analyze the diversity and distribution of testate amoebae in Chile, a country that encompasses the southwestern region of South America, countless islands and part of the Antarctic. In Chile, known diversity includes 416 testate amoeba taxa (64 genera, 352 infrageneric taxa), 24 of which are here reported for the first time. Species–accumulation plots show that in Chile, the number of testate amoeba species reported has been continually increasing since the mid-19th century without leveling off. Testate amoebae have been recorded in 37 different habitats, though they are more diverse in peatlands and rainforest soils. Only 11% of species are widespread in continental Chile, while the remaining 89% of the species exhibit medium or short latitudinal distribution ranges. Also, species composition of insular Chile and the Chilean Antarctic territory is a depauperated subset of that found in continental Chile. Nearly, the 10% of the species reported here are endemic to Chile and many of them are distributed only within the so-called Chilean biodiversity hotspot (ca. 25° S–47° S). These findings are here thoroughly discussed in a biogeographical and evolutionary context.

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Keywords: Amoebozoa; Biogeography; Ecology; SAR supergroup; South America; The Antarctic

Introduction

Testate amoebae are a polyphyletic group of shelled protists present in many terrestrial, freshwater and occasionally marine habitats. Their sensitivity to slight changes in the environmental conditions has turned them into an increasingly

used group of bioindicators to monitor pollution (Nguyen-Viet et al., 2007; Meyer et al. 2012), ecosystem restoration and management (Turner and Swindles 2012; Valentine et al. 2013), cadaver decomposition (Seppey et al. 2015; Szelecz et al., 2013) or natural ecological gradients (Opravilova and Hajek 2006; Payne 2011; Koenig et al. 2015). Their shells can be preserved for a long time in peats and sediments, and ancient communities are nowadays commonly used to monitor past environmental changes (Mitchell et al. 2008; Payne and Pates 2009). To date, testate amoebae have been assigned to two different deep eukaryotic lineages: Amoebozoa (lobose testate amoebae) and the supergroup SAR

*Corresponding author at: Laboratory of Soil Biology, Institute of Biology, University of Neuchâtel, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland.

E-mail addresses: limnoleo@gmail.com, leonardo.fernandez@unine.ch (L.D. Fernández).

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(filose testate amoebae) (Adl et al. 2012). Past estimations of their diversity reached about 2,000 species (Meisterfeld 2002a, 2002b). However, these estimations are currently being revised due to the introduction of DNA barcoding, which revealed an important cryptic diversity (Kosakyan et al. 2012; Heger et al. 2013; Singer et al. 2015). Notably, it has been shown in several cases that allopatric speciation occurred, and several species are known to have limited geographic distributions as demonstrated with both morphological observations (Smith and Wilkinson 2007; Smith et al. 2008; Zapata and Fernández 2008) and molecular methods (Heger et al. 2013).

The study of the diversity and distribution of testate amoebae boasts a long tradition (Penard 1902; Claparède and Lachmann 1868; Ehrenberg 1853; Leidy, 1879). However, most studies on these microorganisms have been developed in the Holarctic region (Foissner 1997, 1999; Smith et al. 2008). Data from the Southern Hemisphere are still rare and sparse, indicating a limited knowledge on these protists along a considerable fraction of the terrestrial surface of the planet. Chile encompasses almost all the southwestern region of South America, countless islands and part of the Antarctic continent and covers a vast array of climatic zones. Therefore, this country has a considerable interest for testate amoebae biogeography. Despite a long history of records of testate amoebae from Chile, the data are still scattered and incomplete. Knowledge of Chilean testate amoebae began with Ehrenberg (1843), who studied a few samples collected at Cape Horn (Horn Island, Chile), the southernmost headland of Tierra del Fuego. Forty-six years later, Certes (1889) reported several new species and genera after studying samples collected from 1882 to 1883 by the French Scientific Mission to Cape Horn at Orange Bay (Hoste Island, Chilean part of Tierra del Fuego). In this manuscript, Certes described remarkable species such as *Apodera vas* (Certes, 1889) and *Certesella martiali* (Certes, 1889). Subsequently, Wailes (1913) added some new records and species from samples collected in Punta Arenas, Valparaíso and Antofagasta from austral, central and northern continental Chile, respectively. It is noteworthy that back then, all mentioned localities were important seaports, which were (or still are) part of international commercial sea routes. This probably largely determined the selection of sampling sites at that time. Later, Jung (1942a) published a paper documenting a large number of known and then unknown taxa that he observed in samples collected both in the Reloncaví Inlet and the inland territory of south-central Chile. This paper was important for the natural history of Chilean testate amoebae as it broke the trend of sampling exclusively at seaports and documented the species that were present in remote and often pristine sites. Some years later, Hoogenraad and de Groot (1951) reported some testate amoeba species that they found in moss samples collected in austral and central Chile. Then, Bonnet (1966) investigated the community composition and distribution of testate amoebae in south-central Chile. He described some new species and found

that the distribution of these organisms was mainly determined by the C:N ratio, pH and water availability in different terrestrial habitats. More recently, Smith (1978, 1985) documented the testate amoeba species composition of the Chilean Antarctic territory, revealing a pauperized diversity. Likewise, Zapata and colleagues further documented the testate amoeba species composition of south-Central Chile (Zapata and Rudolph 1986), the Chilean Antarctic territory (Zapata and Matamala 1987) and Easter Island (Zapata and Crespo 1990). Finally, the 20th century ended with the contribution of Golemansky and Todorov (1996), who described for the first time the psammobiotic fauna from the marine littoral and supralittoral of Chile. The current century has witnessed an increase in the number of studies involving testate amoebae. Several papers describing the Chilean testate amoebae fauna and ecology as well as providing new records or describing new species have been published during the last years (Zapata et al. 2002; Zapata 2005; Zapata et al. 2007a, 2007b; Zapata and Fernández 2008; Zapata et al. 2008; De Smet and Gibson 2009; Fernández and Zapata 2011; Santibañez et al. 2011; Fernández et al. 2012; Chatelain et al. 2013; Fernández 2015).

Bringing together more than 170 years of data accumulated by numerous researchers, this paper represents the first attempt to synthesize the knowledge about Chilean testate amoebae, including both published and unpublished information. The first purpose of this study was to give an updated and comprehensive species checklist that includes nomenclature changes prompted by recent research as well as ecological information and geographical data on the distribution of each species in the whole Chilean territory including continental Chile, insular Chile and the Chilean Antarctic territory. The second purpose of this study was to investigate the diversity and spatial patterns of these organisms in Chile using qualitative and quantitative approaches including estimations of richness at different taxonomic levels, habitats surveyed, habitat preference, latitudinal patterns of distribution, levels of endemism and comparisons among the species composition recorded on insular, Antarctic and continental Chile. We expect that this manuscript will contribute to reduce the existing knowledge gap between South American and Holarctic testate amoebae and encourage the local research interest in this group.

Material and Methods

Geographical setting

Chile is a long and narrow country situated on the Pacific coast of South America, stretching over 4300 km southwards from latitude 17°30' S to Cape Horn at 56° S. Chile also has sovereignty over the Pacific islands of Easter Island and the Juan Fernández Archipelago and claims Antarctic Territory between 53° and 90° W. As a result, Chile is usually partitioned in three political divisions: continental Chile, insular

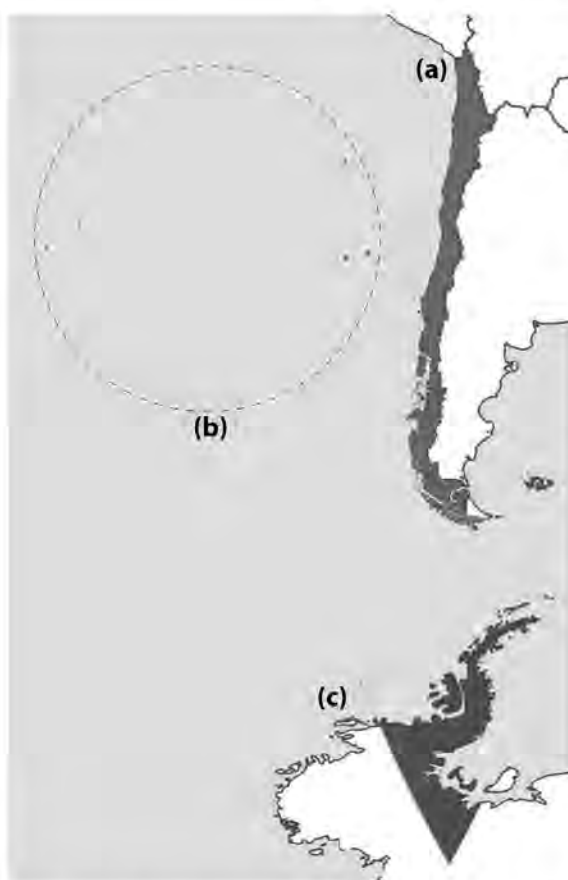


Fig. 1. The Chilean territory includes: (a) continental Chile, which stretches from 17°30' S to Cape Horn at 56° S; (b) insular Chile, which includes many continental and oceanic islands (the latter are indicated by the dotted circle); and (c) the Antarctic territory included between 53° W and 90° W.

Chile and the Chilean Antarctic territory (Fig. 1). Chile is regarded as a land of climatic and geographic extremes. Roughly, the climate of continental Chile is dry and hot in the north, mostly Mediterranean in the central region, and both subpolar and oceanic in the southern region. The climate of insular Chile is either subtropical or oceanic and the Chilean Antarctic territory is dominated by a polar climate. Thus, Chile overall includes many major biomes including hot and cold deserts, alpine, arid shrublands, deciduous forests, rainforests and grasslands. A considerable part of the Chilean territory also represents a biodiversity hotspot called the Chilean Winter Rainfall-Valdivian Forests Hotspot (i.e. the Chilean hotspot) (Arroyo et al. 2004). The Chilean hotspot stretches between 25° S and 47° S, including a narrow coastal strip between 19° S and 25° S, plus the Juan Fernández Archipelago.

Database construction

To construct the database used in this study, we first did an exhaustive literature search that included all published material devoted, partially or completely, to testate amoebae of Chile. Second, we performed a comprehensive review of material stored both in the private collection of Jaime Zapata (a retired Chilean protistologist) and in the protist collection of the Universidad de Los Lagos (Chile). This material consisted altogether of 250 permanent slides and 100 dry moss samples of 5–10 g each collected between 2000 and 2008 all over continental Chile. We examined individually each permanent slide to ensure that all species reported had been properly identified. For moss samples, we took a sub-sample of 3 g of dry moss, re-hydrated it in 250 ml of distilled water and sieved it through 400 and 15 µm mesh sieves. Each fraction was examined under an inverted microscope for documenting species occurrences. The identification of taxa was based on morphological characters of the shell following traditional taxonomic references. Important sources used in the identification of amoebae included those by Certes (1889); Jung (1942a, 1942b); Mazei and Tsyganov (2006) and Zapata et al. (2007a, 2007b). When possible, the amoebae observed were photographed by means of a digital camera connected to an inverted microscope and/or by scanning electron microscopy (SEM). For SEM microscopy, the individuals were cleaned by several transfers through distilled water, mounted on stubs and finally air-dried. The individuals on stubs were coated with gold and photographed with either a JEOL JSM-6380 or Philips XL30 operating between 10 and 15 kV. Third and finally, several species identified from samples collected by the first author between 2007 and 2013 in insular and continental Chile (6275 soil/moss/litter samples of ca. 10 cm³ each) were also integrated into the raw database. The analyses of these samples as well as the isolation and identification of these species followed the same process described above for dry moss samples.

As we compiled data from various sources that were generated using different methods and taxonomical approaches, we needed to correct several spelling errors, misidentifications and obsolete taxon names (e.g. synonyms, homonyms and *nomina nuda* taxa). We also sent our raw database to colleagues who also have taxonomic expertise in the group to have their opinion (see acknowledgments section). Their comments and suggestions were taken into account in the final version of the database.

We thus obtained a comprehensive and updated species checklist for the Chilean testate amoebae. Taxa identified at species level included forms indicated as *confer* (cf.) or with a question mark (?), meaning that organisms were similar to described species but not identical, thus representing probably new species. Intraspecific taxa included those taxa mentioned as subspecies, varieties or forms in the literature. Along with the record of each taxon, we also took note of: (a) its species authority; (b) synonyms used in the Chilean testate amoebae literature; (c) infraspecific taxa used in the Chilean

literature; (d) the habitat(s) where it has been recorded in Chile; (e) additional notes describing for example, the person who first reported/described the taxon in Chile, related homonyms, etc.; (f) its geographical distribution (i.e. whether the species occurs in continental Chile and/or insular Chile and/or the Chilean Antarctic territory); and (g) references (i.e. manuscripts that mention the occurrence of the taxon in the Chilean territory). This additional information was compiled from the consulted literature as well as from our personal observations.

Classification of the species at higher taxonomic levels followed Adl et al. (2012). These authors proposed a hierarchical system of classification founded on robust phylogenetic relatedness that avoid the use of formal rank designations such as “class”, “sub-class”, “super-order”, or “order”. The reason to do so has been motivated by utility, to avoid the common problem of a single change causing a cascade of changes to the system (Adl et al. 2012). The classification of those taxa not included in the revision of Adl et al. (2012) was based on other authors (e.g. Meisterfeld 2002a, 2002b; Haman 1988; Golemansky and Todorov 1996).

Diversity analyses

We used the information compiled in the final database to calculate the total number of valid testate amoeba taxa recorded so far within the whole Chilean territory including genera, species, infraspecific taxa and unidentified species. We then recalculated the total number of testate amoeba taxa only for continental Chile, insular Chile and the Chilean Antarctic territory to assess the differences in diversity among these territories. All these calculations were also done separately on amoebozoan and SAR testate amoebae. We then constructed a summary table for these data. In addition, we counted the number of species included in each amoebozoan and SAR genus to assess their contribution to the overall diversity. We also constructed species accumulation curves calculated in function of time to document the rate of species description over the years in Chile. Finally, we assessed the completeness of the inventory of Chilean testate amoeba species richness using the samples collected by the first author (see database construction) as a reference sample to compute sample-size-based rarefaction and extrapolation species richness curves (see Colwell et al. 2012). The extrapolation curve was calculated up to double the size of the reference sample. Rarefaction and extrapolation curves, as well as their 95% confidence intervals (bootstrap method, 20,000 replications), were computed using R 3.1.0 and the *iNEXT* package (Hsieh et al. 2014).

Habitats explored and habitat preference

We investigated which habitats have been explored to study the Chilean testate amoebae. To do this, we looked at our database and listed all the habitats reported there. Then, we

classified each habitat as terrestrial, aquatic and semi-aquatic. Here, we also introduced a new type of semi-aquatic habitat locally known as Mallín (Mallines in plural). A Mallín (from the Mapudungun language = flooded place) is a wetland that can be roughly described as a swampy grassland with mineral soils. This type of habitat is common in southern Chile, southern Argentina and their adjacent islands (Schlatter and Schlatter 2004). A Mallín remains wetter than the adjacent soils in summer and is commonly flooded during winter, favoring the concentration of organic matter and the development of wetland vegetation such as plants of the genera *Acaena*, *Azorella*, *Festuca* and *Juncus*. Depending on the structure of the soil, a Mallín sometimes can even harbor medium-sized native trees (Schlatter and Schlatter 2004). While a Mallín can also contain some isolated *Sphagnum* patches, it differs from a peatland in that it has a completely different soil composition and pH, among other physico-chemical characteristics. Refer to Fig. S1 for an example of a Mallín. Table S1 to see some of the physicochemical properties that characterize and differentiate a Mallín from a peatland. We finally counted the number of species that have been recorded in each habitat as a measure of habitat preference for these protists, where habitat preference is the use of some habitats over others by the organisms (Hall et al. 1997). This information was also explored for Amoebozoa and SAR and then put in a summary table.

Biogeographical aspects

To evaluate latitudinal patterns in the distributions of testate amoeba species, we divided continental Chile into 1° latitudinal bands (i.e. 39 latitudinal bands), registering the presence or absence of each species in each latitudinal band. The presence–absence data were used to construct a binary matrix with columns as latitudinal bands and rows as taxa. Maps of species distributions were created using data with a spatial resolution of 1° of latitude. Ranges were assumed to be continuous between latitudinal bands. To describe latitudinal patterns of species, we categorized species distribution ranges into three groups: (a) species with small distributions, occurring only within two latitudinal bands; (b) species with small–medium ranges of distribution, ranging from three to 10 latitudinal bands; (c) species with medium–large distributions, ranging from 11 to 19 latitudinal bands; and (d) species with large distributions, ranging from 20 to 39 latitudinal bands.

To determine if the taxa observed in Chile were characterized by a wide or more restricted geographical distribution, we classified the species as: (a) widely distributed, if they occurred in more than one continent; (b) South American, if they only occurred in South America; (c) Austral, if they only occurred in the southern part of Chile and Argentina (ca. 40° S to 56° S); and (d) endemics, if they only have been recorded in Chile.

Moreover, it has been proposed that the testate amoeba species composition of insular Chile and the Chilean

Antarctic territory is only a subset of that present in continental Chile (Smith 1985; Zapata and Matamala 1987; Zapata and Crespo 1990; Fernández et al. 2012). To test this hypothesis, we performed a nested analysis on the binary matrix mentioned above. Nested analysis predicts the existence of a highly ordered system in which taxa composition in depauperated sites tend to be simple subsets of richer ones (i.e. a nested pattern) (Wright et al. 1998; Fernández 2015). We used the method of Atmar and Patterson (1993) and the temperature metric (T°) to explore the occurrence of a nested pattern. The temperature metric evaluates the degree of disorder as estimated by the temperature of a binary matrix, where $T^\circ = 0^\circ$ indicates perfect nestedness and $T^\circ = 100^\circ$ indicates complete randomness. Before calculating the degree of nestedness, the binary matrix was sorted according to the marginal row and column sums, with common taxa placed in the upper rows and taxa-rich latitudinal bands placed in the left-hand column (Ulrich et al. 2009; Fernández 2015). Islands and the Antarctic continent were treated as having a direct borderline with continental Chile. We also calculated the degree of nestedness only for Amoebozoa and then only for SAR. The statistical significance of these analyses was evaluated with a row–column null-model (50,000 permutations, $p < 0.05$) a conservative algorithm that minimizes Type I errors (Gotelli 2000). Nestedness and null-model calculations as well as temperature matrices were done using R 3.1.0 and the *vegan* package (Oksanen et al. 2013).

Results

Analyses on testate amoeba diversity

During the analysis of the raw database, we identified two homonyms genera, transferred seven species to other genera (*combinatio nova*), synonymized 83 specific and infraspecific taxa, and identified four invalid species (*nomen nudum* taxa). All these homonyms, transferred, synonymized and invalid taxa are properly identified in the species checklist (Tables S2 and S3). After these procedures, the total number of valid testate amoeba taxa recorded in Chile reached 352 infrageneric taxa distributed in 64 genera (Table 1). From these taxa, 24 species (17 amoebozoan and 7 SAR) are here reported for the first time for Chile (Tables S2 and S3). When

we recalculated the total number of taxa for each of the three Chilean political divisions, we found that continental Chile is much more diverse than insular Chile and the Chilean Antarctic Territory (Table 1). In addition, when we calculated the number of taxa for Amoebozoa and SAR, we found that the first group is much more diverse than the second, both in the whole Chilean territory and in any of its political divisions (Table 1, Tables S2 and S3).

Among Amoebozoa, *Diffugia* and *Centropyxis* are the most diverse genera with 48 (18% of the total) and 41 (16%) taxa, respectively (Fig. 2a). Other genera such as *Arcella* (23 taxa, 9%), *Nebela* (18 taxa, 7%), *Cyclopyxis* (15 taxa, 6%), *Heleopera* (14 taxa, 6%) and *Argyria* (11 taxa, 4%) also made an important contribution to the Amoebozoa diversity (Fig. 2a). These genera together accounted for 66% of the total amoebozoan testate amoeba diversity recorded in Chile. The other 29 genera accounted for the remaining 34% of the total amoebozoan testate amoeba diversity (Fig. 2a).

Euglypha was the most diverse genus of SAR testate amoebae with 32 taxa (34% of the total) (Fig. 2b). Other species-rich genera were *Corythion* (seven taxa, 8%), *Pseudodiffugia* (seven taxa, 8%), *Sphenoderia* (six taxa, 7%) and *Trinema* (11 taxa, 5%). Together these five genera represented about 61% of the total SAR testate amoeba diversity (Fig. 2b). The other 23 genera made a low contribution to the whole diversity as together they accounted for no more than 39% of the total diversity reported in Chile (Fig. 2b).

The counting of the species richness within each genus also revealed large gaps in species identification. At least 37 testate amoeba species (11% of the total) remain unidentified. The gaps in species identification are particularly important in Amoebozoa genera such as *Arcella* (22%) and *Phryganella* (40%), as well as in SAR genera such as *Euglypha* (13%) and *Pseudodiffugia* (57%).

The assessment of the evolution of the number of species recorded as a function of time showed that the recorded and described diversity of testate amoebae in Chile has been continually increasing over time without leveling off (Fig. 3a). This analysis revealed an increase of over 20% (64 taxa) in the number of new taxa reported for Chile since the year 2000 (i.e. 15 years). This pattern was recorded for both Amoebozoa and SAR, with increases of 21 (49 taxa) and 17% (15 taxa), respectively (Fig. 3b, c). The assessment of the completeness of the inventory of Chilean testate amoeba species richness

Table 1. Total number of generic and infrageneric valid testate amoeba taxa (TA), Amoebozoa taxa (AMO) and SAR taxa recorded in Chile and its three political divisions.

Taxon	Chile			Continental Chile			Insular Chile			Antarctic territory		
	TA	AMO	SAR	TA	AMO	SAR	TA	AMO	SAR	TA	AMO	SAR
Genera	64	36	28	64	36	28	14	12	2	13	10	3
Species	255	184	71	252	181	71	32	28	4	23	14	9
Intraspecific taxa	60	46	14	57	43	14	4	4	0	0	0	0
Unidentified species	37	27	10	30	21	9	5	5	0	2	1	1

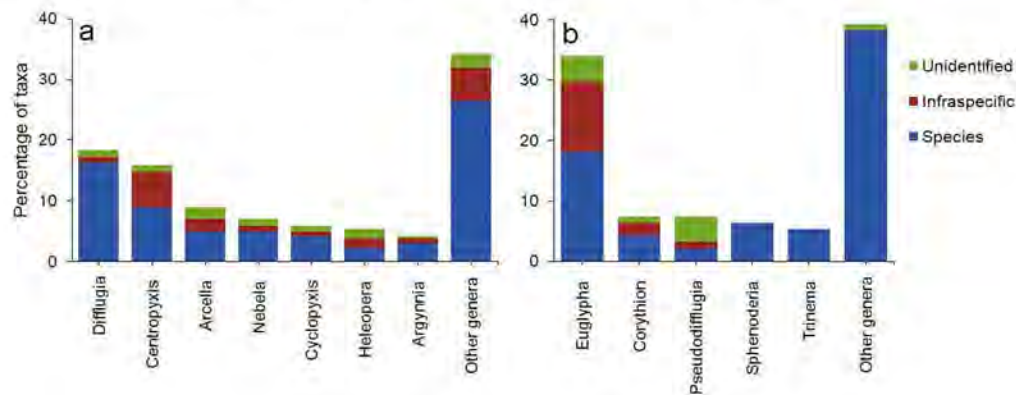


Fig. 2. Percentage of infra-generic taxa included in each (a) amoebozoan genus and (b) SAR genus in Chile. Only the most diverse genera (those that made up over 60% of the total diversity either in Amoebozoa or SAR) are detailed.

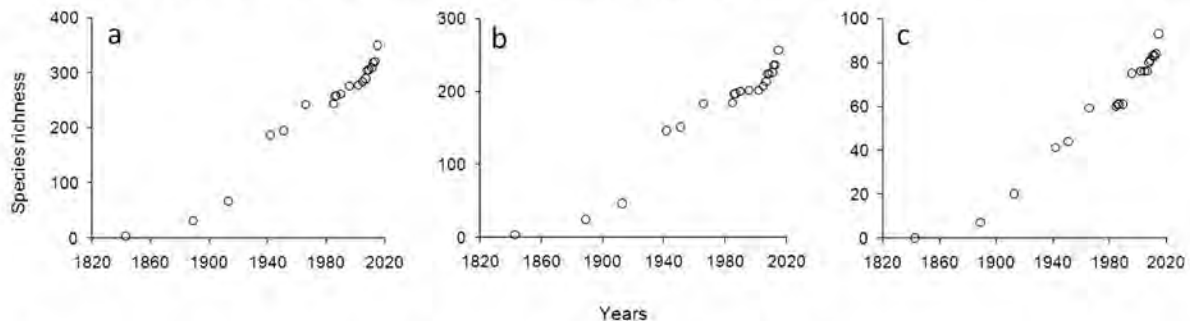


Fig. 3. Species accumulation curves calculated in function of time for: (a) all testate amoebae, (b) amoebozoan testate amoebae, and (c) SAR testate amoebae.

showed a steep slope for the rarefaction curve, indicating that the current sampling effort (i.e. the reference sample) has not been enough to detect the whole species diversity. This analysis also showed a steep slope for the extrapolation curve, suggesting that Chilean testate amoeba species diversity is far from complete, even if we double the current sampling effort (Fig. 4).

Habitats explored and habitat preference

A thorough analysis of the database constructed for this study revealed that testate amoebae were recorded from 37 different habitats (Table 2). Of these, 17 habitats were classified as terrestrial, 12 as aquatic, and six as semi-aquatic. Most testate amoeba species were found in semi-aquatic (peatlands, 110 species) and terrestrial (rainforest soils [litter], 94 species) habitats (Table 2). Amoebozoan testate amoebae were commonly found in aquatic (pond sediments, 93 species) and semi-aquatic (peatlands, 91 species) habitats, whereas SAR testate amoebae were more often documented in terrestrial (rainforest soils [litter], 35 species) and semi-aquatic (peatlands, 20 species) habitats (Table 2, Tables S2 and S3).

Biogeographical analyses

When analyzing the latitudinal patterns of the species, we found that only a small fraction exhibit widespread distribution along continental Chile. Of the 252 testate amoebae

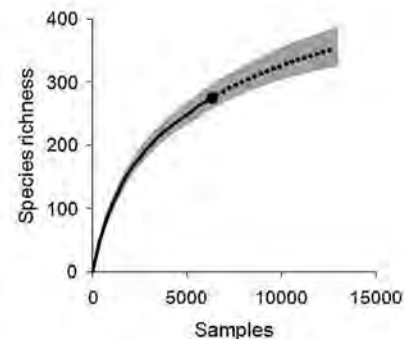
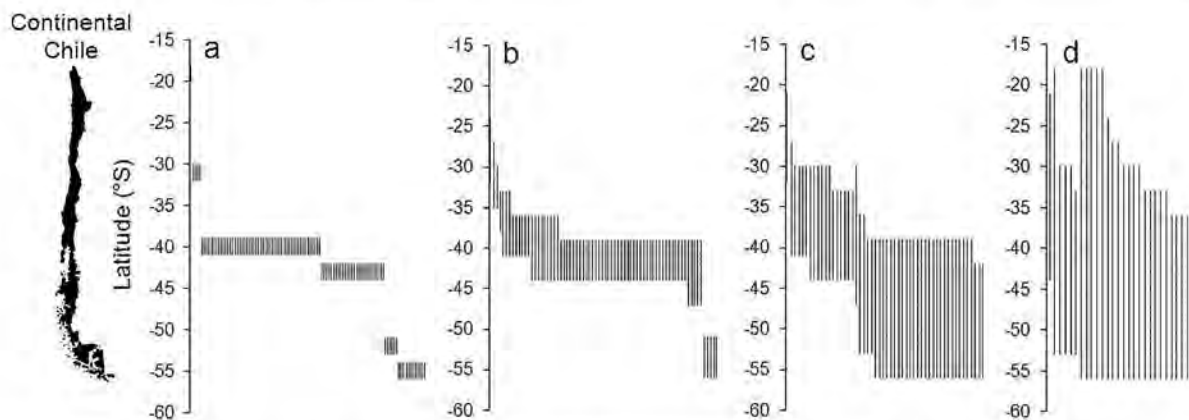


Fig. 4. Size-based rarefaction (solid curve) and extrapolation (dotted curve) with 95% confidence intervals (shaded area, based on a bootstrap method with 20,000 replications). The reference sample is indicated by a solid black dot.

Table 2. Habitats explored to study the Chilean testate amoebae (TA), including amoebozoan testate amoebae (AMO), SAR testate amoebae, and the total number of species that have been recorded in each habitat.

Type	Habitat	TA	AMO	SAR
Terrestrial	Acid mixed soils	14	6	8
	Arid shrublands	25	16	9
	Conifer forest soils (native)	9	3	6
	Humic soil lawns	26	16	10
	Humus in trunks	24	15	9
	Humus on trunks	20	14	6
	Magellanic rainforest soils	9	5	4
	Mosses and lichens covered by snow	9	0	9
	Mosses on trees (living trees)	7	3	4
	Mosses on trunks (dead trees)	15	9	6
	Muddy soils	20	15	5
	North Patagonian rainforest soils	19	19	0
	Rainforest mosses	29	21	8
	Rainforest soils (litter)	94	59	35
	Soil humus	14	11	3
	Soil mosses	85	61	24
	<i>Sphagnum</i> mosses	34	21	13
Aquatic	Estuaries	20	19	1
	Forest streams	64	50	14
	Freshwater column	10	5	5
	Glacial rivers	7	4	3
	Hot springs	6	3	3
	Lake sediments	16	13	3
	On aquatic plants	33	27	6
	On freshwater algae	13	10	3
	Pond sediments	93	77	16
	River sediments	25	24	1
	Stream sediments	52	47	5
	Volcanic crater lakes	12	7	5
Semi-aquatic	Mallines	27	22	5
	Peatlands	110	91	20
	Saltmarshes	10	9	1
	Sandy supralittoral	17	2	15
	Swamps	72	61	11
	Volcanic crater glaciers	4	0	4

**Fig. 5.** Latitudinal distribution patterns recorded for 252 testate amoeba species in continental Chile. (a) 102 species (40% of the total) have small distributions; (b) 70 species (28%) exhibit small–medium ranges of distribution; (c) 52 species (21%) have medium–large distributions; and (d) 28 (11%) species have large distributions. Each vertical band represents the distribution of a single species.

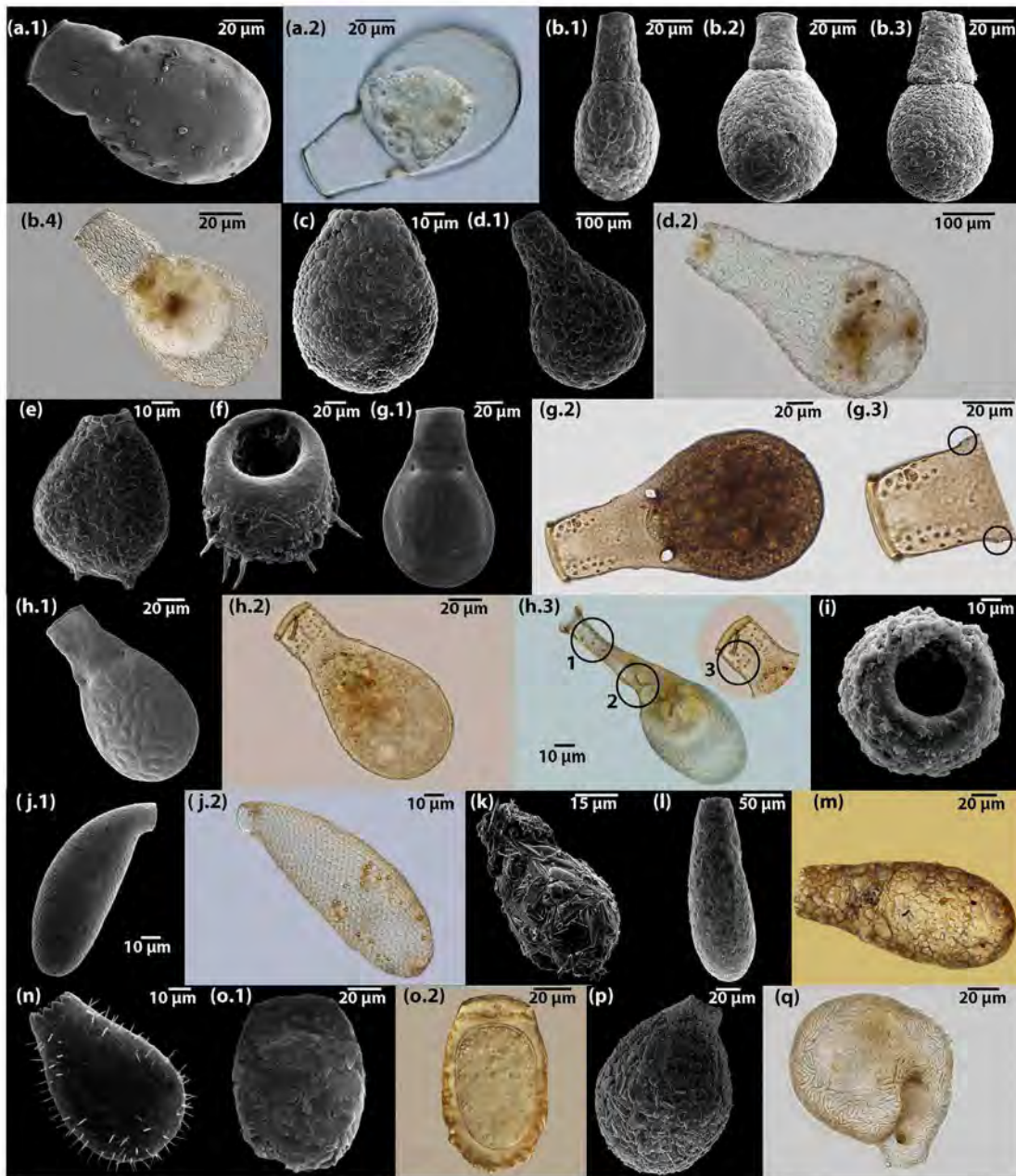


Fig. 6. Scanning electron microscopy and light micrographs of typical testate amoeba taxa found in Chile: (a) *Alocodera cockayni* (Penard, 1910): (a.1) from a peatland, TDF; and (a.2) living individual from a rainforest, SC. (b) *Apodera vas* Jung, 1942: (b.1–b.3) three different morphotypes that co-occur in peatlands of CC (from Zapata and Fernández 2008); and (b.4) living individual from a peatland, TDF. (c) *Argynnia dentistoma* (Penard, 1890): individual from a rainforest, IC (from Fernández et al. 2012). (d) *A. gertrudeana* Jung, 1942: (d.1) individual from a swamp, SC; and (d.2) living individual from a rainforest, CC. (e) *A. schwabei* Jung, 1942: from a peatland, CC. (f) *Centropyxis aculeata* (Ehrenberg, 1838): individual from a hot spring, SC. (g) *Certesella martiali* (Certes, 1889): (g.1) individual from a Mallín, CC; (g.2) living individual from a peatland, TDF; and (g.3) close-up of the neck of the previous individual, showing both the two rows of internal teeth that run along the neck (giving it a dotted appearance) and the small lateral pores that are usually present in this taxon (circles). (h) *C. certesi* (Penard, 1911): (h.1) individual from soil mosses, SC; (h.2) living individual from a peatland, TDF; (h.3) lateral view of the previous individual, showing the internal teeth of the neck (circle 1), the internal tube that connect the two big and invaginated pores that characterized the genus

species that have been recorded in continental Chile: (a) 102 species (40% of the total) exhibit restricted distributions, occurring only within two latitudinal bands (Fig. 5a); (b) 70 species (28%) exhibit small–medium ranges of distribution, ranging from three to 10 latitudinal bands (Fig. 5b); (c) 52 species (21%) exhibit medium–large distributions, ranging from 11 to 19 latitudinal bands (Fig. 5c); and (d) 28 (11%) species exhibit large distributions, ranging from 20 to 39 latitudinal bands (Fig. 5d).

Furthermore, a high number of species occurring in Chile exhibit a wide geographical distribution although an important proportion also appears as unique to southern Chile. In detail, we determined that: (a) 269 species (86% of the total) are widely distributed; (b) three species (1%) are restricted to South America; (c) 11 species (3%) are austral; and (d) 31 species (10%) are endemic to Chile. A further analysis revealed that almost all the endemic species are restricted to south-central Chile an area included within the so-called Chilean hotspot. The endemism recorded is due to the high number of unique amoebozoan and SAR testate amoeba taxa present in this region, which, respectively, exceeds 10 and 8% of the total number of species recorded in Chile. The taxa that are potentially endemic to South America, to austral South America and Chile are highlighted in the additional notes section of Tables S2 and S3. Figs 6 and 7 show SEM and light micrographs of some of these potentially endemic taxa and other typical taxa found in Chile. It is noteworthy that these figures show for the first time some species that were previously only known from poor quality drawings or photographs. These images thus confirm their existence.

The temperature metric confirmed that insular and Antarctic species composition represents a subset of that present in continental Chile at different taxonomic criteria for all testate amoebae, for amoebozoan taxa, and for SAR taxa (Table 3). However, a few exclusive taxa have also been recorded in both insular Chile and the Chilean Antarctic territory (Tables S2 and S3).

Discussion

In Chile, testate amoeba diversity is very high (Table 1), with a magnitude comparable to or even higher than in other regions of the planet. The Chilean testate amoeba diversity appears two or three times higher than that of other Neotropical countries, such as Peru, Colombia, Ecuador or Mexico (Haman and Kohl 1994; Escobar et al. 2005; Krashevskaya et al.

2007; Bobrov and Krasilnikov 2011). The testate amoeba diversity recorded in Chile even exceeded that recorded in Argentina or China, countries that historically have experienced a sampling effort comparable to that observed in Chile (Vucetich and Lopretto 1995; Qin et al. 2011). However, none of these countries have been systematically sampled through all its biomes in the way we sampled Chile. Still, when compared with well-studied regions of the world, Chilean testate amoeba diversity stands high. A recent revision, (Smith et al. 2008) shown that so far 229 species have been recorded in North and Central America, 428 in Australia, 648 in Africa and 1031 in Europe. Thus, the Chilean diversity is exceeded only by that recorded in Australia, Africa and Europe. Note, however, that we are comparing here the diversity observed in Chile with magnitudes of diversity recorded in whole continents. Moreover, we do not know to what extent these comparisons could be valid because as stressed by Smith et al. (2008), the higher diversity recorded for some regions might be only a reflection of intensity of sampling, rather than true higher diversity. Chile, for instance, has only started to be sampled systematically in the middle of the twentieth century, while European countries have been surveyed during more than two centuries (Foisner 1997, 1999). In turn, it is probable, for instance, that countries such as Colombia that are acknowledged to contain diversity hotspots for many (macroscopic) taxa (Myers et al. 2000) harbor an even larger diversity than Chile. However, a representative area of Chile also is regarded as a biodiversity hotspot (Arroyo et al. 2004), and some studies have highlighted the independent origin of its diversity (Segovia and Armesto 2015). Further surveys will perhaps confirm whether Chilean diversity surpasses other under-sampled regions. In any case, the number of species recorded in Chile has increased over the time. Our study indeed suggests that the number of species recorded for Chile will continue increasing, as the evolution of the species number recorded as a function of time have not yet reached a plateau (Fig. 3). Likewise, sample-size-based rarefaction and extrapolation curves show that previous studies have overlooked a large fraction of testate amoeba species diversity and that many species still remains undiscovered (Fig. 4).

The diversity of testate amoebae was much higher in continental Chile than in the Chilean Antarctic territory and insular Chile (Table 1). This finding is in agreement with the observation of several authors who have reported that the species composition found in both the Chilean Antarctic territory and insular Chile is only a depauperated subset of that found in

Certesella (circle 2), and unusual internal lateral teeth (circle 3). (i) *Cyclopyxis eurytoma* Deflandre, 1929: individual from an estuary, CC. (j) *Cyphoderia ampulla* (Ehrenberg, 1840): (j.1) individual found in a pond, CC; (j.2) living individual from soil mosses, SC. (k) *Diffugia bacillifera* Penard, 1890: individual from a peatland, CC. (l) *D. lanceolata* Penard, 1890: individual from a stream, IC (from Fernández et al. 2012). (m) *D. oblonga* Ehrenberg, 1838: living specimen found in a peatland, SC. (n) *Euglypha strigosa* (Ehrenberg, 1871): individual from an arid shrubland, NC. (o) *Heleopera petricola* Leidy, 1879: (o.1) individual of *H. petricola major* Cash, 1909 found in a rainforest, CC; and (o.2) a representative of the typical form found in a peatland, Tierra del Fuego. (p) *Lesquereusia modesta* Rhumbler, 1895: found in a peatland, CC. (q) *L. spiralis* (Ehrenberg, 1840): found in a pond, SC. Figures (d) and (e) are the first pictures ever taken for these taxa. NC: northern Chile; CC: central Chile; SC: southern Chile; TDF: Tierra del Fuego; IC: insular Chile.

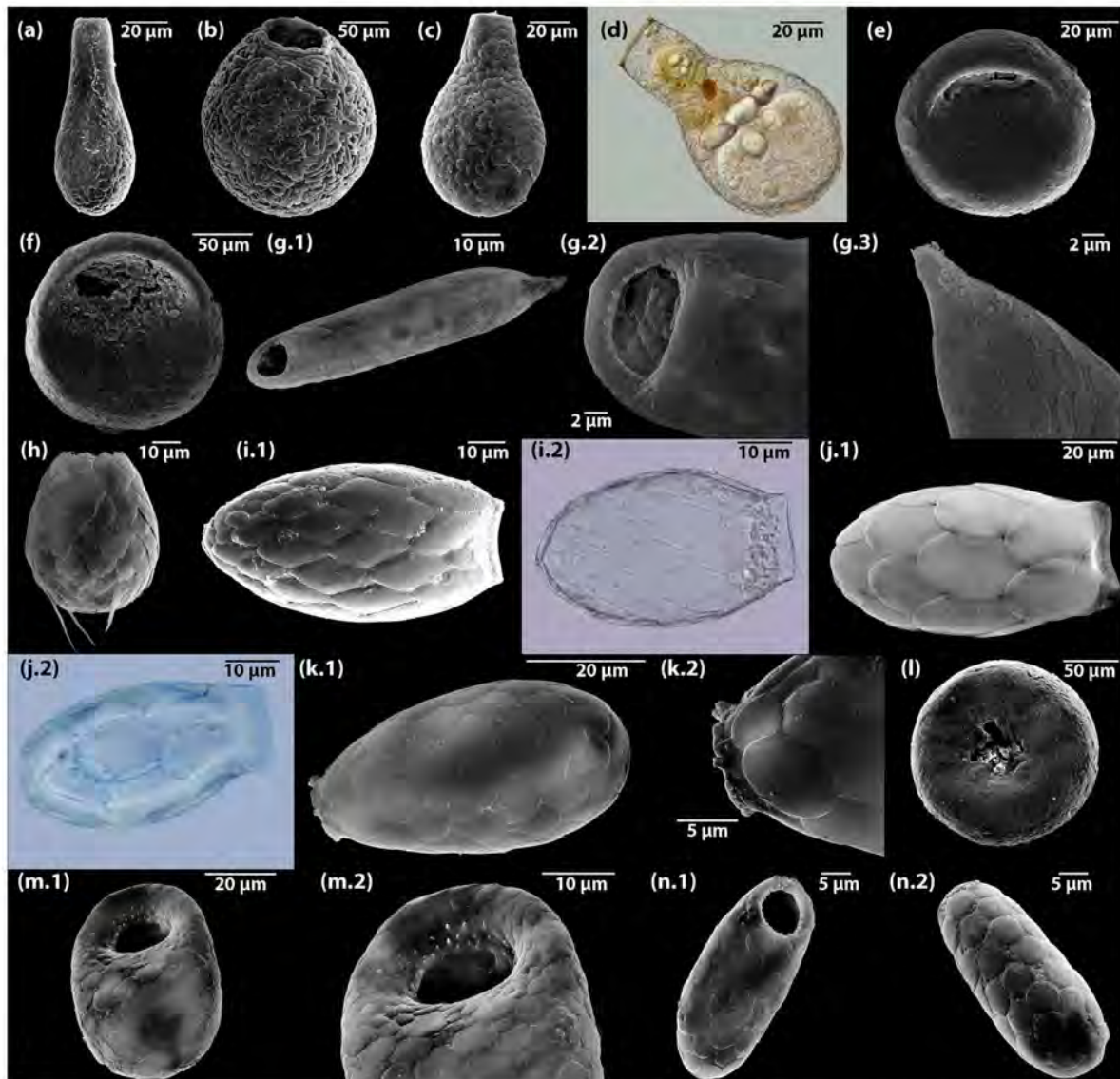


Fig. 7. Scanning electron microscopy and light micrographs of typical testate amoeba taxa found in Chile: (a) *Nebela barbata psilonata* Jung, 1942: from a rainforest, IC (from Fernández et al. 2012). (b) *Netzelia wailesi* (Ogden, 1980): from an arid shrubland, NC (from Fernández 2015). (c) *Padaungiella lageniformis* (Penard, 1902): from a rainforest, IC (from Fernández et al. 2012). (d) *P. wailesi* (Deflandre, 1936): living specimen from soil humus, CC. (e) *Plagiopyxis callida* Penard, 1910: from soil mosses, CC. (f) *P. labiata* Penard, 1911: found in humus, CC. (g) *Puytoracia jenswendti* Santibañez et al., 2011: (g.1) individual from a volcanic crater glacier, SC; (g.2) oral aperture; and (g.3) detail of the aboral horn or spine (from Santibañez et al. 2011). (h) *Scutiglypha cabrolae* De Smet and Gibson, 2009: from an arid shrubland, NC (from Fernández 2015). (i) *Sphenoderia rhombophora* Bonnet, 1966: (i.1) and (i.2) show the same individual which was found in a forest dominated by *Pilgerodendron uviferum*. (j) *S. valdiviana* Chatelain et al., 2013: (j.1) individual from a rainforest, SC; (j.2) living individual from soil mosses, SC. (k) *Tracheleuglypha dentata* (Penard, 1890): (k.1) individual from an arid shrubland, NC; and (k.2) detail of the oral aperture. (l) *Trigonopyxis arcuata* (Leidy, 1879): from an estuary, SC. (m) *Trinema complanatum* Penard, 1890: (m.1) individual from an arid shrubland, NC (from Fernández 2015); and (m.2) detail of the oral aperture. (n) *Trinema lineare* Penard, 1890: individual found in soil mosses, (n.1) frontal view; and (n.2) posterior view. Figure (i), is the first SEM picture ever taken for this taxon. NC: northern Chile; CC: central Chile; SC: southern Chile; TDF: Tierra del Fuego; IC: insular Chile.

Table 3. Degree to which species-poor communities inhabiting insular Chile and the Chilean Antarctic territory are subsets of species-rich communities located in continental Chile. Nestedness was based on a presence–absence data matrix ordered according to the marginal row/column sums. Below are shown the observed and expected values of the temperature metric for all testate amoebae, for testate amoebae belonging to the Amoebozoa and for testate amoebae belonging to the SAR. Statistical inference was based on the lower and upper confidence limits (L95% CL and U95% CL, respectively) of the null distribution of 50,000 randomized matrices.

	Temperature metric		L95% CL	U95% CL	<i>p</i> -value
	Observed	Expected			
Testate amoebae	1.74	6.83	1.97	12.73	0.001
Amoebozoa	0.95	5.32	2.53	10.64	0.001
SAR	0.82	6.27	3.12	11.52	0.001

continental Chile (Smith 1985; Zapata and Matamala 1987; Zapata and Crespo 1990; Fernández et al. 2012). Our nested analyses underpinned this trend by showing the existence of a nested pattern of species composition between these three territorial divisions (Table 3). However, this does not mean that exclusive, and thus, potentially endemic species are absent in the Antarctic and insular territories. Indeed, several species have only been recorded in these regions while others could not be identified to species level, suggesting that these territories could harbor an unexplored diversity (Fernández et al. 2012). The low diversity recorded in the Antarctic can be attributed mainly to the existence of hostile environmental conditions and lack of suitable habitat for the establishment of viable populations (Smith 1985; Zapata and Matamala 1987). However, this cause cannot be used to explain the low diversity in insular Chile. Chilean islands include habitats similar to those found in continental Chile, which in turn are potentially suitable for the establishment of testate amoebae (Zapata and Crespo 1990; Fernández et al. 2012).

Among the possible causes for the low insular diversity are the remoteness and the area of the islands. According to the theory of island biogeography (MacArthur and Wilson 1963), taxa diversity is a decreasing function of the isolation of the island and an increasing function of the area of the island. Thus, taxa composition in insular Chile must be a pauperized subset of that recorded in the continent because first, only good dispersers may colonize islands; and second, the area of the islands represents a small fraction of the continent. However, while the area per se probably does not directly determine the protist species richness of islands, it probably determines the heterogeneity of habitat (e.g. pH, temperature and moisture ranges) and thus, the number of inter-specific interactions that occur on islands (Hutchinson 1957). Besides the above prediction, the airborne dispersal hypothesis (Wilkinson 2001; Lara et al. 2011; Wilkinson et al. 2012) predicts that the small species (those having a size of below a certain threshold, such as 100 or 20 μm) are more likely to be passively dispersed over long distances than large species. Although the mechanism proposed by this hypothesis could be a surrogate of other physiological traits of protists (e.g. perhaps, larger species are worse dispersers because they have more labile cysts) (Fernández et al. 2012) it arises as a

potential cause in our study. Continental islands (i.e. islands that lie on the continental shelf) are closer to continental Chile and indeed contain several large taxa (Fernández et al. 2012), while oceanic islands (i.e. islands that do not sit on the continental shelf) are farther from the continent and are almost free of large taxa (Zapata and Crespo 1990).

Moreover, when compared with continental Chile, the diversity of insular Chile and the Chilean Antarctic territory is abysmally low (Table 1). We suggest that this pattern is, in part, an artifact of the method used to identify the species in these territories. There, species have been historically identified with a morphological approach. This approach does not allow discriminating between pseudocryptic and cryptic species and therefore may underestimate protist diversity. We think that the use of molecular approaches to identify species in these territories would help to detect various pseudocryptic and cryptic species, increasing the known number of species for insular Chile and the Chilean Antarctic territory. However, by saying this we are not asserting that the use of molecular approaches will show that these territories are equally or more diverse than continental Chile. Most likely, diversity in these territories would still be largely a subset of that found in continental Chile (e.g. as predicted by the theory of island biogeography). Nonetheless, the use of molecular approaches certainly would show that these territories also harbor endemic species that do not co-occur in continental Chile and that they are not as poor in species as shown by the current data.

As a general rule, we recorded higher testate amoeba diversity within the Amoebozoa than within the SAR in Chile and all its political divisions (Table 1). Amoebozoan testate amoebae mainly include large taxa (e.g. *Certesella*, *Nebela*), while SAR testate amoebae are mostly represented by very small taxa (e.g. *Trinema*, *Sphenoderia*) (Meisterfeld 2002a, 2002b). Thus, it is not clear whether this is a real trend (e.g. Amoebozoa exhibits higher diversification rates) or only a bias introduced by the fact that very small species are often overlooked or hardly differentiated. In support for the latter explanation, a recent environmental DNA survey revealed in forest litter samples the presence of Paulinellidae; a taxon of SAR largely overlooked by conventional methods in terrestrial habitats (Tarnawski and Lara 2015). Likewise, detailed (SEM) morphological analyses have shown that

small difference in scaling patterns can discriminate sometimes quite genetically divergent species of SAR (Wylezich et al. 2002; Chatelain et al. 2013).

Our analyses showed that Chilean testate amoebae are very diverse in semi-aquatic or terrestrial habitats such as peatlands and rainforest soils (Table 2). These habitats are quite common and abundant at mid-latitudes (Gajardo 1994; Schlatter and Schlatter 2004), coinciding with areas where there is a constant tradeoff between water and solar energy inputs throughout the year (i.e. temperate areas) (Arroyo et al. 2004). The ecological conditions that prevail in these areas certainly favor the occurrence of a high diversity, since water availability and mild temperatures are particularly critical for the survival and reproduction of testate amoebae (Meisterfeld 2002a, 2002b; Fernández 2015). In fact, the geographical distribution of most species is restricted to the temperate zone, and few species extend its geographical distribution beyond mid-latitudes (Fig. 5). Moreover, our analyses showed that some representatives of these protists can also occur in hot and dry habitats or cold and dry habitats such as arid shrublands and glaciers (Table 2). These habitats are common at low and high latitudes (Gajardo 1994; Zapata and Matamala 1987), where there is a constant deficit of water or solar energy inputs over the year, respectively (Arroyo et al. 2004). Because of these characteristics, these habitats are considered suboptimal for testate amoebae (Smith 1985; Zapata and Matamala, 1987; Fernández 2015). This lead us to suggest that populations living in these habitats are not self-sustaining and therefore, are supported by continual immigration of individuals coming from optimal habitats (temperate areas), where populations are actively growing and reproducing (i.e. a source-sink dynamics at biogeographical scale, see Fernández 2015). The fact that most of the species that occurs at low and high latitudes also occur at mid-latitudes supports this idea (i.e. protist species composition at low and high latitudes are mainly a subset of that found at mid-latitudes) (Fig. 5).

Our study also revealed that one-third (33%) of testate amoeba species recorded in Chile are widely (20–38 latitudinal bands) or relatively widely (11–19 latitudinal bands) distributed all along continental Chile while other were reported over shorter latitudinal ranges (Fig. 4). Species exhibiting vast geographical distributions all along Chile are often also generalists with wide ecological tolerances to various environmental factors, such as *Arcella vulgaris* or *Centropyxis aculeata*. The first species can tolerate high salt concentrations due to leaching from de-iced roads (Roe and Patterson 2014), and can live in activated sludge (Jaromir-Glen et al. 2013) as well as in acidic *Sphagnum* mosses environment (Mieczan 2007). The second occurs from alkaline lakes (Qin et al. 2013) to Amazonian peatland pools (Swindles et al. 2014), tolerates also well organic pollution (Dorham et al. 2013) and can survive in arid soils that are dry for much of the year (Fernández 2015). Because of their tolerance, they have a high colonization potential; these species are considered as cosmopolitan.

However, most widespread species have never been studied with molecular methods. An exception here is *Nebela collaris*, which has been found to be rather a complex of species than a single entity, encompassing nowadays at least eight “morpho-phylogenetic species” (Singer et al. 2015). It is therefore possible that seemingly widespread species comprise several entities with more restricted distributions. A similar case occurs with some relatively widespread taxa such as *Apodera vas* and *Certesella* spp. These taxa are consistently distributed in southern and central Chile but their latitudinal distribution is abruptly cut northward by the so-called ‘South American arid diagonal’, where climatic conditions become drier (ca. 30° S; southern edge of the Atacama Desert). They exhibit high morphological variation within and between populations along their latitudinal distributions, and morphological parameters measured show significant discontinuities (Zapata and Fernández 2008; Fig. 6). This suggests that these taxa represent species complexes consisting of several closely related species with more restricted distributions than *A. vas* and *Certesella* spp. sensu lato. Moreover, species exhibiting restricted geographical distributions in Chile are both very rare and medium- to large-sized taxa (ca. 70–250 µm) that inhabit specific habitats, such as *Argynnia schwabei*, *A. gertrudeana*, *Puytoracia jenswendtii* and *Scutiglypha cabrolae* (Figs 6 and 7). The first two species are only found within habitats included in the southern rainforest biome (Zapata 2005; Fernández and Zapata 2011), the third has been found only on the surface of a glacier (Santibañez et al. 2011) and the fourth is only known from an isolated high altitude volcanic lake in the Andes Range (De Smet and Gibson 2009) and extremely arid soils of the southern edge of the Atacama Desert (Fernández 2015). Most of the species with restricted geographical distributions are also endemic to Chile and their distributions are within the Chilean biodiversity hotspot. The value of endemism recorded so far for Chile reaches close to 10% of the total diversity. Some researchers believe that a value close to 10% is sufficient for an area to qualify as a geographical province, at least for macroscopic organisms (Briggs and Bowen 2012). Therefore, the Chilean biodiversity hotspot could potentially host a unique micro-eukaryotic diversity and represent an area of evolutionary innovations or refuges where an older and still understudied microbiota persists. We trust that future molecular-based studies and phylogeographic analyzes will enable us to test this hypothesis.

Conclusions

The Chilean territory represents an area of high diversity for testate amoebae. In this country, testate amoeba diversity has exponentially increased in recent years and this trend will continue over time. This conjecture is supported by the emerging use of molecular techniques, which promise to detect a cryptic diversity largely overlooked by traditional approaches. Testate amoebae also comprise a widespread

group in Chile that occurs in many habitats, though they are more diverse in peatlands and rainforest soils. However, this pattern may be an artifact of uneven sampling effort, since in Chile the latter habitats have been more sampled than others. This calls for studying other less explored habitats (e.g. hot and cold deserts, glaciers) to expand our view on its ecology thus allowing us to use them as bioindicators of the past and present-day environmental conditions in this region of the planet.

Testate amoebae are not randomly distributed in Chile. Species composition of Insular Chile and the Chilean Antarctic territory is mainly a depauperated subset of that found in continental Chile. This nested pattern of species composition has been also found for several metazoans and has been explained in terms of the theory of island biogeography. We suggest that the occurrence of this pattern also is driven by the same mechanisms proposed by this theory (i.e. selective extinction and selective colonization).

Moreover, in continental Chile only generalist species are widely distributed (ca. 11% of the total), occurring from temperate to extremely hot and cold biomes. In contrast, the remaining 89% of the species have a much more restricted distribution, occurring mainly in the temperate zone where there is a constant tradeoff between water and mild temperatures inputs over the year. At least the 10% of these species are potentially endemic to Chile and occur exclusively in temperate biomes of the so-called Chilean biodiversity hotspot. These diversity patterns have been repeatedly found for Chilean metazoans and have been explained in terms of ecological, historical and evolutionary causes. For example, the lift of the Andes and the Pleistocene glaciations changed the climate in this region, creating hot and cold climates at high and low latitudes, respectively. These changes forced the species to flee into mid-latitudes (temperate areas), where they undergone evolutionary events that resulted in new species. Then, since hot and cold climate conditions still prevail at low and high latitudes, very few species (mainly generalist species) have been able to re-colonize these areas (Arroyo et al. 2004). We suggest that these processes also determined the spatial distribution of these protists in continental Chile.

Finally, the occurrence of the aforementioned diversity patterns suggests that, in Chile, testate amoebae and metazoans exhibit analogous diversity patterns driven by similar underlying mechanisms. Future biogeographical, macroecological and phylogeographic analyses may validate this idea.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ejop.2015.07.001>.

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Appendix to “Checklist, diversity and distribution of testate amoebae in Chile”

Fernández L.D., Lara E., Mitchell E.A.D., Checklist, diversity and distribution of testate amoebae in Chile. *Eur. J. Protist.* (2015), <http://dx.doi.org/10.1016/j.ejop.2015.07.001>

SUPPORTING INFORMATION



Fig. S1 A Mallín from south-central Chile dominated by native wetland plants and trees. This picture was taken in summer 2008. In this region the annual precipitation is ca. 4000 mm per year and therefore, this habitat can be completely flooded during winter. Photo credits: L.D. Fernández.

Fernández L.D., Lara E., Mitchell E.A.D., Checklist, diversity and distribution of testate amoebae in Chile. Eur. J. Protist. (2015), <http://dx.doi.org/10.1016/j.ejop.2015.07.001>

SUPPORTING INFORMATION

Table S1 Physicochemical differences between a Mallín and a peatland (modified from Roig and Roig 2004).

	Mallín	Peatland
Soil type	Mineral soil (Mollisol)	Organic soil (Histosol)
Organic matter (%)	< 20-35	> 20-35
Organic carbon (%)	< 12-20	> 12-20
pH	Neutral	Acid
Apparent density	High	Low
Porosity (%)	Low (45-55)	High (80)
Hydraulic conductivity	High except clays	Low to High
Field capacity	Low	High
Available nutrients	Usually high	Usually low
Cation exchange capacity	Lower dominated by major cations	High, dominated by hydrogen ions
Typical vegetation	<i>Acaena</i> , <i>Azorella</i> , <i>Festuca</i> <i>Carex</i> , <i>Juncus</i>	<i>Astelia</i> , <i>Donatia</i> , <i>Sphagnum</i>

Reference

Roig C. and Roig F.A., 2004. Capítulo 1: Consideraciones generales, In: Blanco, D.E. and de la Balze V.M. (Eds.), *Los Turbales de la Patagonia: Bases para su inventario y la conservación de su biodiversidad*. Publicación No. 19, Wetlands International; Buenos Aires, Argentina, 5–21.

SUPPORTING INFORMATION

Table S2 Checklist of amoebozoan taxa recorded in Chile. The classification of the species at higher taxonomic levels follows (except when noticed otherwise) that of Adl et al. (2012). Only the synonyms, subspecific taxa and habitats mentioned in the Chilean literature are listed. The 'Additional notes section' shows extra information for each species. The 'Distribution section' shows the geographical distribution of each species, where the abbreviations indicate: NC (northern continental Chile), CC (central continental Chile), SC (southern continental Chile), EI (Easter Island), GI (Guamblin Island), AN (the Chilean Antarctic territory). The 'References section' shows the studies that report each species in Chile.

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
• Tubulinea	Smirnov et al. 2005						
•• Arcellinida	Kent 1930						
••• Arcellina	Hässel 1804						
•••• Arcellidae	Ehrenberg, 1843						
Arcella	Ehrenberg, 1832						
<i>Arcella angulata</i>	(Playfair, 1917)	No	No	Lakeshores	This species was first recorded by Zapata and Matamala (1987)	AN	Zapata and Matamala (1987)
<i>Arcella arenaria</i>	Grouff, 1966	No	No	Arid shrublands, mosses on trees, peatlands, pond sediments, soil mosses, stream sediments	This species was first recorded by Wailes (1913)	NC, CC, SC	Wailes (1913); Hooijmaad and de Groot (1951); Fernández (2015)
<i>Arcella artocrea</i>	Leidy, 1879	No	No	Peatlands	This species was first recorded by Zapata et al. (2008)	CC, SC	Zapata et al. (2008); Fernández and Zapata (2011)
<i>Arcella calinus</i>	Pennard, 1890	No	No	On aquatic plants	This species was first recorded by Zapata and Rudolph (1986)	SC	Zapata and Rudolph (1986)
<i>Arcella discoides</i>	Ehrenberg, 1843	No	<i>A. discoides</i> <i>pseudovulgaris</i> Delfandere, 1928; <i>A. discoides</i> <i>scutelliformis</i> Playfair, 1918	Glacial rivers, lakeshores, on aquatic plants, on freshwater algae, peatlands, pond sediments, rainforest mosses, soil mosses, Sphagnum mosses, swamps	This species was first reported by Wailes 1913. Jung (1942) also reported the following subtaxa: <i>A. discoides pseudovulgaris</i> Delfandere, 1928 and <i>A. discoides scutelliformis</i> Playfair, 1918	CC, SC	Wailes (1913); Jung (1942); Zapata and Rudolph (1986); Zapata et al. (2005)
<i>Arcella excavata</i>	Cunningham, 1919	No	No	Peatlands	This species is first reported for Chile in the present study	CC	Present study
<i>Arcella gibbosa</i>	(Pennard, 1890)	<i>A. vulgaris gibbosa</i> Pennard, 1890	No	Mallines, peatlands, pond sediments, stream sediments, soil mosses, Sphagnum mosses	This species was first recorded by Wailes 1913 under the name of <i>Arcella vulgaris gibbosa</i> Pennard, 1890	CC, SC	Wailes (1913); Jung (1942); Zapata et al. (2007a); Zapata et al. (2008); Fernández and Zapata (2011)
<i>Arcella hemisphaerica</i>	Petty, 1869	No	<i>A. hemisphaerica undulata</i> Delfandere, 1928	Lakeshores, muddy soils, on aquatic plants, pond sediments, rainforest streams, volcanic crater lakes, swamps	This species was first reported by Jung (1942) under the following names: <i>A. hemisphaerica undulata</i> Delfandere, 1928 and <i>A. hemisphaerica undulata</i> . Note that <i>A. hemisphaerica undulata</i> is a misspelled version of <i>A. hemisphaerica undulata</i> . <i>A. hemisphaerica undulata</i> was also reported by Zapata and Matamala (1986)	CC, SC, EI, AN	Jung (1942); Zapata and Rudolph (1986); Zapata and Matamala (1987); Zapata and Crespo (1990)
<i>Arcella intermedia</i>	(Delfandere, 1928)	<i>A. hemisphaerica intermedia</i> Delfandere, 1928	<i>A. intermedia laevis</i> (Delfandere, 1928)	On aquatic plants, stream sediments	This species was first reported by Zapata and Rudolph (1986) as <i>A. hemisphaerica intermedia</i> . However, this subtaxa is a junior synonym of the subspecific taxon <i>A. intermedia laevis</i> (Delfandere, 1928) (see Mazei and Tsyganov 2006)	CC	Zapata and Rudolph (1986)
<i>Arcella irregularis</i>	Molz, 1961	No	No	Rainforest soils, soil mosses	This species is first reported in the present study	SC	Present study
<i>Arcella megastoma</i>	Pennard, 1902	No	No	Pond sediments, soil mosses, stream sediments	This species was first recorded by Wailes (1913)	SC	Wailes (1913)
<i>Arcella rotundata</i>	Playfair, 1918	No	<i>A. rotundata aplanata</i> Delfandere, 1928	Hot springs, on aquatic plants, on freshwater algae, pond sediments, rainforest soils	This species was first reported by Jung (1942) under the name of <i>A. rotundata aplanata</i> Delfandere	CC, SC	Jung (1942); Zapata and Rudolph (1986)
<i>Arcella vulgaris</i>	Ehrenberg, 1830	No	No	Arid shrublands, humid soil lawns, Mallines, on aquatic plants, peatlands, pond sediments, saltmarshes, soil mosses	This species was first recorded by Wailes (1913)	NC, CC, SC, AN	Wailes (1913); Jung (1942); Bonnet (1966); Smith (1978); Zapata and Rudolph (1986); Zapata (2005); Fernández and Zapata (2011); Fernández (2015)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Arcella</i> sp. 1		No	No	Soil mosses	This taxon was reported by Jung (1942) from soil moss samples collected in south-central Chile	CC	Jung (1942)
<i>Arcella</i> sp. 2		No	No	Pond sediments	This taxon was reported by Jung (1942) from pond sediment samples collected in south-central Chile	CC	Jung (1942)
<i>Arcella</i> sp. 3		No	No	Pond sediments	This taxon was reported by Jung (1942) from pond sediment samples collected in south-central Chile	CC	Jung (1942)
<i>Arcella</i> sp. 4		No	No	Soil mosses	This taxon was reported by Hoogenraad and de Groot (1951) from soil moss samples (<i>Campylopus introflexus</i> [Hedw.]) collected in south-central Chile	CC	Jung (1942)
<i>Arcella</i> sp. 5		No	No	Malines	This taxon was reported by Zapata et al. (2007a) from samples collected in Malines, Puyehue National Park, south-central Chile	CC	Zapata et al. (2007a)
.... Microchlamyidae							
Microchlamys							
<i>Microchlamys patella</i>	Oudem. 1888 Cockerell, 1911 (Claparède and Lachmann, 1859)	<i>Pseudochlamys patella</i> Claparède and Lachmann, 1859	No	Forest streams, peatlands, swamps	This species was first reported by Jung (1942) as <i>Pseudochlamys patella</i> Claparède and Lachmann, 1859	CC	Jung (1942)
.... Microcoryciidae							
Diplochlamys							
<i>Diplochlamys fragilis</i>	Giesl, 1988	No	No	Humus in trunks, mosses on trunks	This species was first reported by Bonnet (1966)	CC	Bonnet (1966)
<i>Diplochlamys limda</i>	Perard, 1900	No	No	Humus in trunks, rainforest soils	This species was first reported by Bonnet (1966)	CC	Bonnet (1966)
Microcorycia							
<i>Microcorycia flava</i>	Cockerell, 1911 (Giesl, 1988)	No	No	Humic soil lawns	This species was first reported by Bonnet (1966)	CC	Bonnet (1966)
Parmulina							
<i>Parmulina cyathus</i>	Perard, 1902	No	No	Stream sediments, lakeshore mosses, pond sediments, <i>Sphagnum</i> mosses, swamps	This species was first reported by Jung (1942)	CC, SC	Jung (1942)
<i>Parmulina oblecta</i>	(Culver, 1883)	No	No	Pond sediments	This species was first reported by Jung (1942)	CC, SC	Jung (1942)
... Diptugina							
.... Centropxyidae							
Centropxyis							
<i>Centropxyis aculeata</i>	Stein, 1857 (Ehrenberg, 1888)	No	<i>C. aculeata aculeata</i> (Ehrenberg, 1841), <i>C. aculeata grandis</i> Delandere, 1929, <i>C. aculeata grandis</i> Delandere, 1929, <i>C. aculeata oblonga</i> Delandere, 1929	Arid shrublands, forest streams, hot springs, humid soil lawns, lake sediments, lakeshore mosses, Malines, muddy soils, North Patagonian rainforest soils, on freshwater algae, on aquatic plants, peatlands, pond sediments, saltmarshes, stream sediments, soil mosses, river sediments, <i>Sphagnum</i> mosses, swamps	This species was first reported by Walles (1913) together with the subtaxa <i>C. aculeata ecoris</i> (Ehrenberg 1841) and <i>C. aculeata hemisphaerica</i> (Barnard, 1875). Currently these subtaxa have attained the species status (see <i>C. ecoris</i> and <i>C. hemisphaerica</i>). Later Jung (1942) also reported the subtaxa <i>C. aculeata oblonga</i> Delandere, 1929 and <i>C. aculeata grandis</i> Delandere, 1929. Fernández et al. (2012) also mentioned <i>C. aculeata aculeata</i> (Ehrenberg, 1841) and <i>C. aculeata oblonga</i> Delandere, 1929	NC, CC, SC, AN, GI	Walles (1913), Jung (1942), Bonnet (1966); Zapata and Rudolph (1986); Zapata et al. (2005); Zapata et al. (2007a); Zapata et al. (2008); Fernández and Zapata (2011); Fernández et al. (2012); Fernández (2015)
Centropxyis aerophila							
<i>Centropxyis aerophila</i>	Delandere, 1929	No	<i>C. aerophila cornuta</i> Delandere, 1929, <i>C. aerophila peltiformis</i> Bonnet, 1966, <i>C. aerophila</i> on trunks, lakeshores, mosses on trunks, North Patagonian rainforest soils, on freshwater algae, on aquatic plants, peatlands, pond sediments, rainforest mosses, rainforest soils, <i>Sphagnum</i> mosses, soil mosses, swamps	Arid shrublands, estuaries, forest streams, humid soil lawns, humus in trunks, humus on trunks, lakeshores, mosses on trunks, North Patagonian rainforest soils, on freshwater algae, on aquatic plants, peatlands, pond sediments, rainforest mosses, rainforest soils, <i>Sphagnum</i> mosses, soil mosses, swamps	This species was first reported by Jung (1942)	NC, CC, SC, AN, GI	Jung (1942); Bonnet (1966); Zapata and Matamala (1987); Zapata et al. (2002); Zapata (2005); Zapata et al. (2007a); Zapata et al. (2008); Fernández and Zapata (2011); Fernández et al. (2012); Fernández (2015)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Centropogon cassis</i>	(Wallich, 1864)	No	No	Acid mixed soils, estuaries, forest streams, glacial rivers, hot springs, lakeshores on aquatic plants, on freshwater algae, pond sediments, rainforest mosses, rainforest soils, river sediments, soil mosses, <i>Sphagnum</i> mosses, swamps	This species was first reported by Jung (1942)	CC, SC	Jung (1942); Bonnet (1966); Zapata and Rudolph (1986); Zapata et al. (2002)
<i>Centropogon compressa</i>	(Jen Oye, 1948)	No	No	Peatlands, soil mosses	This species is first reported for Chile in the present study	CC, SC	Present study
<i>Centropogon constricta</i>	(Ehrenberg, 1841)	<i>Diffugia constricta</i> Ehrenberg, 1841	<i>C. constricta minima</i> Declaire, 1954; <i>C. constricta</i> 'a' Zapata et al., 2008; <i>C. constricta</i> 'b' Zapata et al., 2008	Arid shrublands, forest streams, humid soil lawns, humus in trunks, lakeshores, lake sediments, Magellanic rainforest soils, mosses on trees, muddy soils, North Patagonian rainforest soils, on aquatic plants, peatlands, pond sediments, rainforest mosses, rainforest soils, river sediments, saltmarshes, soil mosses, stream sediments, swamps, volcanic crater lakes	This species was first reported by Certes under the name of <i>Diffugia (Arcella)</i> <i>constricta</i> . Later Walles (1913) reported this species under the name of <i>Diffugia constricta</i> . Bonnet (1966) also mentioned the subspecific taxon <i>C. constricta minima</i> Declaire; and Zapata et al. (2008) mentioned <i>C. constricta</i> 'a' and 'b'	NC, CC, SC, EI, AN	Certes (1889); Walles (1913); Hogenraad and de Groot (1951); Jung (1942); Smith (1978); Bonnet (1966); Zapata and Rudolph (1986); Zapata and Matanala (1987); Zapata and Crespo (1990); Zapata (2005); Zapata et al. (2007a); Fernández and Zapata (2011); Fernández et al. (2012); Fernández (2015)
<i>Centropogon delicatula</i>	(Pérez, 1902)	No	<i>C. delicatula major</i> Jung, 1942	On freshwater algae, stream sediments	This species was first reported by Jung (1942) together with the first description of the subspecific taxon <i>C. delicatula major</i> Jung (1942).	CC, SC	Jung (1942)
<i>Centropogon discoides</i>	(Pérez, 1900)	No	No	Arid shrublands, forest streams, North Patagonian rainforest soils, peatlands, swamps	This species was first reported by Zapata (2005)	NC, CC, SC, GI	Zapata (2005); Fernández and Zapata (2011); Fernández et al. (2012); Fernández (2015)
<i>Centropogon ecoris</i>	(Ehrenberg, 1841)	<i>C. aculeata ecoris</i> Ehrenberg, 1841	<i>C. ecoris paradoxa</i> Jung, 1942; <i>C. ecoris</i> 'a' Zapata et al., 2008; <i>C. ecoris</i> 'b' Zapata et al., 2008	Estuaries, humid soil lawns, lakeshores mosses, Malines, on aquatic plants, peatlands, pond sediments, river sediments, soil mosses, <i>Sphagnum</i> mosses, swamps	This species was first reported by Walles (1913) under the name of <i>C. aculeata ecoris</i> Ehrenberg 1841. Later Jung described <i>C. ecoris paradoxa</i> Jung, 1942	CC, SC	Walles (1913); Jung (1942); Bonnet (1966); Zapata and Rudolph (1986); Zapata et al. (2002); Zapata (2005); Zapata et al. (2007a); Zapata et al. (2008); Fernández and Zapata (2011)
<i>Centropogon elongata</i>	(Pérez, 1880)	No	No	Acid mixed soils, forest streams, humus in trunks, muddy soils, North Patagonian rainforest soils, peatlands, rainforest soils, stream sediments, swamps	This species was first reported by Bonnet 1966	CC, SC, GI	Bonnet (1966); Zapata (2005); Fernández and Zapata (2011); Fernández et al. (2012)
<i>Centropogon globulosa</i>	(Bonnet and Thomas, 1955)	No	No	Mosses on trunks, soil mosses	This species was first reported by Bonnet (1966)	CC	Bonnet (1966)
<i>Centropogon hemisphaerica</i> (Barnard, 1875)	<i>C. aculeata hemisphaerica</i> Barnard, 1875 (misspelled name of <i>C. hemisphaerica</i>)	No	No	Pond sediments, stream sediments	This species was first reported by Walles (1913) under the name of <i>C. aculeata hemisphaerica</i> Barnard, 1875. Note that <i>C. aculeata hemisphaerica</i> is a misspelled version of <i>C. aculeata hemisphaerica</i>	NC	Walles (1913)
<i>Centropogon hirsuta</i>	(Declaire, 1929)	No	No	Peatlands, pond sediments, river sediments, <i>Sphagnum</i> mosses, stream sediments	This species was first reported by Jung (1942)	CC	Jung (1942); Zapata and Rudolph (1986); Zapata (2005); Fernández and Zapata (2011)
<i>Centropogon laevigata</i>	(Pérez, 1900)	No	No	Lakeshore mosses, on aquatic plants, peatlands, pond sediments, rainforest soils, stream sediments, swamps	This species was first reported by Jung (1942)	CC, SC	Jung (1942); Bonnet (1966)
<i>Centropogon lata</i>	(Jung, 1942)	No	No	Estuaries, lakeshore mosses, pond sediments, stream sediments	This species was first described by Jung (1942) from southern Chile. This species also has been recorded in the northern hemisphere (Törck 1988)	CC, SC	Jung (1942); Zapata et al. (2002)
<i>Centropogon magdalene</i>	Certes, 1889	No	No	Soil mosses	This species was first described by Certes (1889) from samples collected in Tierra del Fuego. Nonetheless this author did not provide a formal and complete description of this species. Certes, planned to publish the formal description and drawings of this species in the journal Mémoires de la Société Zoologique de France (see Certes, 1889). To our knowledge the description of this species was never published. Therefore we consider this species as <i>nomen nudum</i>	SC	Certes (1889)
<i>Centropogon marsupiformis</i>	(Wallich, 1804)	No	No	Soil mosses	This species is first reported in the present study	SC	Present study

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Centropxyxis minuta</i>	(Dalland, 1929)	No	No	Rainforest mosses, soil mosses	This species was first reported by Jung (1942)	CC, SC	Jung (1942)
<i>Centropxyxis notoryx</i>	Jung, 1942	No	No	Lakeshore mosses, pond stream sediments	This species was first described by Jung (1942) from south-central Chile. This species might be considered as endemic to southern Chile as so far it only has been recorded in this geographical region	CC	Jung (1942)
<i>Centropxyxis oblonga</i>	(Dalland, 1929)	No	No	Peatlands	This species is first reported in the present study	CC, SC	Present study
<i>Centropxyxis orbicularis</i>	(Dalland, 1929)	No	No	Glacial rivers, humus in trunks, rainforest soils	This species was first reported by Jung (1942)	CC, SC	Jung (1942); Bonnet (1966)
<i>Centropxyxis plagiosoma</i>	(Bonnet and Thomas, 1965)	No	No	Arid shrublands, humid soil lawns, rainforest soils	This species was first reported by Bonnet (1966)	NC, CC	Bonnet (1966); Fernández (2015)
<i>Centropxyxis platystoma</i>	(Renard, 1890)	No	No	Forest streams, on freshwater algae, peatlands, pond sediments	This species was first reported by Jung (1942)	CC, SC	Jung (1942)
<i>Centropxyxis sylvatica</i>	(Dalland, 1929)	<i>C. verticillata</i> (Dalland, 1929) <i>Centropxyxis sylvatica</i> Bonnet and Thomas, 1965	No	Humus in trunks, humus on trunks, peatlands, rainforest soils, soil mosses	This species was first reported by Jung (1942) as <i>C. aerophila sylvatica</i> Dalland, 1929. Besides <i>C. sylvatica</i> (Dalland, 1929), Bonnet (1966) also reported the occurrence of <i>C. sylvatica minor</i> Bonnet and Thomas, 1955	CC, SC	Jung (1942); Bonnet (1966); Zapata (2005); Fernández and Zapata (2011)
<i>Centropxyxis vandell</i>	(Bonnet, 1966)	No	<i>C. vandell globulosa</i> Bonnet, 1958	Soil mosses	This species was first reported by Bonnet (1966)	CC	Bonnet (1966)
<i>Centropxyxis</i> sp. 1		No	No	Soil mosses	This taxon was reported by Jung (1942) from soil moss samples collected in south-central Chile	CC	Jung (1942)
<i>Centropxyxis</i> sp. 2		No	No	Soil mosses	This taxon was reported by Jung (1942) from soil moss samples collected in south-central Chile	CC	Jung (1942)
<i>Centropxyxis</i> sp. 3		No	No	Soil mosses	This taxon was reported by Jung (1942) from soil moss samples collected in south-central Chile	CC	Jung (1942)
....							
Diffugiidae	Wullich, 1964						
Cucurbitella	Renard, 1902						
<i>Cucurbitella tricuspis</i>	(Carte, 1886)	No	No	Lake sediments, Mallines, saltmarshes, swamps	This species was first reported by Zapata et al. (2007a). Note that this taxon is considered a confusing taxon. According to Medici and Scott (1983) the morphology of this species corresponds with all the species from the genus <i>Neizelia</i> plus <i>Diffugia lobostoma</i> and <i>D. gramen</i> and several other species with lobed aperture	CC	Zapata et al. (2007a)
<i>Cucurbitella</i> sp.		No	No	Mallines	This taxon was reported by Zapata et al. (2007a) from samples collected in Mallines, Puyehue National Park, south-central Chile	CC	Zapata et al. (2007a)
Diffugia	Leclerc, 1915						
<i>Diffugia acuminata</i>	Ehrenberg, 1838	<i>Acropyxis acuminata</i> Jung, 1942	No	Estuaries, lake sediments, peatlands, pond sediments, river sediments	This species was first reported by Jung (1942) under the name of <i>Acropyxis (Diffugia) acuminata</i> Jung, 1942	CC, SC	Certes (1889); Jung (1942); Zapata and Rudolph (1986); Zapata et al. (2002); Zapata (2005); Fernández and Zapata (2011)
<i>Diffugia amphoralis</i>	Hopkinson, 1908	No	No	Peatlands, saltmarshes	This species is first reported in the present study	CC	Present study
<i>Diffugia antarctica</i>	Ehrenberg, 1843	No	No	Soil mosses	This species was first described by Ehrenberg from soil mosses collected in Cabo de Hornos southern Chile. According to Wailes (1913) this species correspond to a representative of the genus <i>Euglyphis</i> . But he adds that the description of this species is so poor that it is impossible to make a robust conclusion. Wailes, suggests considering this species as <i>nomen nudum</i> and here, we have followed his recommendation. This species has not been reported since its first description	SC	Ehrenberg (1948)
<i>Diffugia bacillariarum</i>	Perfy, 1846	No	No	River sediments	This species was first reported by Zapata and Rudolph (1986)	CC	Zapata and Rudolph (1986)
<i>Diffugia bacillifera</i>	Renard, 1890	No	No	Peatlands	This species was first reported by Zapata (2005)	CC	Zapata (2005); Fernández and Zapata (2011)

Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Diffugia bryophila</i>	(Penard, 1902) No	No	Estuaries, forest streams, lake sediments, North Patagonian rainforest soils, on aquatic plants, peatlands, pond sediments, soil mosses, stream sediments, swamps	This species was first reported by Jung (1942)	CC, SC, GI	Jung (1942); Zapata et al. (2002); Zapata (2005); Fernández and Zapata (2011); Fernández et al. (2012)
<i>Diffugia compressa</i>	(Lalby, 1976) No	No	Peatlands	This species is first reported in the present study	CC, SC	Present study
<i>Diffugia corona</i>	Wallich, 1864 No	No	Estuaries, lake sediments, Mallines, peatlands, pond sediments, river sediments, swamps	This species was first mentioned by Certes (1889)	CC, SC	Certes (1889); Zapata and Rudolph (1986); Zapata et al. (2002); Zapata (2005); Zapata et al. (2007a)
<i>Diffugia cylindrus</i>	(Thomas, 1953) No	No	Forest streams, North Patagonian rainforest soils, stream sediments, swamps	This species was first reported by Fernández et al. (2012)	CC, SC, GI	Fernández et al. (2012)
<i>Diffugia cyphodera</i>	(Jung, 1942) No	No	Estuaries, swamps	This species was first described by Jung (1942) from samples collected in southern Chile under the name of <i>D. oblonga cyphodera</i> Jung, 1942.	CC, SC	Jung (1942); Zapata et al. (2002)
<i>Diffugia difficilis</i>	Thomas, 1954 No	No	River sediments	This species was first reported by Zapata and Rudolph (1986)	CC	Zapata and Rudolph (1986)
<i>Diffugia distenda</i>	(Penard, 1899) No	No	Arid shrublands, forest streams, peatlands, stream sediments, swamps	This species was first reported by Jung (1942) under the name of <i>Acipyxis acuminata inflata</i> (Penard, 1899). This species also has been reported as <i>D. mifloriformis</i> Wallich, 1864. However, a detailed examination of the pictures enclosed in those papers reporting the occurrence of this taxon revealed that, in fact, the species observed was <i>D. distenda</i> (Penard, 1899)	NC, CC, SC, GI	Jung (1942); Zapata and Rudolph (1986); Zapata et al. (2002); Zapata (2005); Fernández and Zapata (2011); Fernández et al. (2012); Fernández (2015)
<i>Diffugia elegans</i>	Penard, 1930 No	No	Mallines, peatlands, pond sediments, river sediments, swamps	This species was first reported by Jung (1942) under the name of <i>Acipyxis elegans</i> (Penard, 1899)	CC, SC	Jung (1942); Zapata et al. (2007a); Zapata et al. (2008)
<i>Diffugia erinacea</i>	(Jung, 1942) No	No	Forest streams, lakeshores, peatlands, swamps	This species was first described by Jung (1942) from samples collected in southern Chile under the name of <i>Acipyxis (Diffugia) erinacea</i> Jung (1942). <i>D. erinacea</i> Jung, 1942 might be considered as endemic to southern Chile as so far it only has been recorded in this geographical region	CC, SC	Jung (1942)
<i>Diffugia gigas</i>	(Jung, 1942) No	No	Forest streams	This species was first described by Jung (1942) from samples collected in southern Chile under the name of <i>Pleurodiffugia gigas</i> Jung, 1942. <i>D. gigas</i> Jung, 1942 might be considered as endemic to southern Chile as so far it only has been recorded in this geographical region	CC, SC	Jung (1942)
<i>Diffugia glans</i>	Penard, 1902 No	No	Peatlands, pond sediments, rainforest mosses, swamps	This species was first described by Jung (1942) from samples collected in southern Chile under the name of <i>Pycnochila glans</i> Jung, 1942	CC, SC	Jung (1942); Zapata (2005); Fernández and Zapata (2011)
<i>Diffugia globularis</i>	Wallich, 1864 No	No	Estuaries, on aquatic plants, stream sediments, swamps	This species was first reported by Zapata and Rudolph (1986)	CC, SC, GI	Zapata and Rudolph (1986); Zapata et al. (2002); Fernández et al. (2012)
<i>Diffugia globulosa</i>	Dujardin, 1837 No	No	Lake sediments, peatlands, pond sediments	This species was first reported by Certes (1889)	CC, SC	Certes (1889); Zapata et al. (2005); Fernández and Zapata (2011)
<i>Diffugia globulus</i>	(Ehrenberg, 1848) No	No	Pond sediments, soil mosses, <i>Sphagnum</i> mosses, stream sediments, swamps	This species was first reported by Jung (1942) as <i>Loboramina globulus</i> (Ehrenberg, 1848), and then as <i>Globonata globulus</i> (Ehrenberg, 1848)	CC, SC	Jung (1942)
<i>Diffugia golmanskyi</i>	Vucelich, 1972 No	No	Peatlands	This species was first reported by Fernández and Zapata 2011. This species was first described by Vucelich (1972) from samples collected in southern Argentina. This species is potentially endemic to southern South America (southern Chile plus southern Argentina) as it only has been recorded in this geographical area	CC	Fernández and Zapata (2011)
<i>Diffugia granen</i>	Penard, 1902 No	No	Pond sediments, soil mosses, stream sediments	This species was first reported by Walles (1913)	8C	Walles (1913)

Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Diffugia hermitana</i>	Ehrenberg, 1843	No	Soil mosses	This species was first described by Ehrenberg from soil mosses collected in Cabo de Hornos southern Chile. According to Wallies (1913) the description of this species is so poor that it is impossible to make a robust conclusion about its taxonomic validity. This author suggested that the individuals observed by Ehrenberg could have corresponded to representatives of the species <i>Cortesia catesi</i> (Penard, 1911). Wallies, suggests considering this species as <i>nomine nudum</i>	SC	Wallies (1913)
<i>Diffugia hisrio</i>	Penard, 1908	No	Forest streams, swamps	This species was first reported by Jung (1942)	CC, SC	Jung (1942)
<i>Diffugia immanata</i>	Acropyxis <i>immanata</i> Jung, 1942	No	Forest streams	This species was first described by Jung (1942) as <i>Acropyxis inflata immanata</i> Jung (1942) from samples collected in southern Chile. This species is potentially endemic to central and southern Chile	CC, SC	Jung (1942)
<i>Diffugia lacustris</i>	Penard, 1899	No	Peatlands, river sediments, swamps	This species was first reported by Jung (1942)	CC, SC	Jung (1942); Zapata and Rudolph (1986); Zapata (2005); Zapata et al. (2008); Fernández and Zapata (2011)
<i>Diffugia laevis</i>	Penard, 1902	No	Forest streams, pond sediments, river sediments, <i>Sphagnum</i> mosses	This species was first reported by Jung (1942) under the name of <i>Cingodiffugia laevis</i> (Penard 1902). Ouden (1978), suggested that this taxon could belong to the genus <i>Neizella</i>	CC	Jung (1942); Zapata and Rudolph (1986)
<i>Diffugia lanceolata</i>	Penard, 1890	No	Muddy soils, North Patagonian rainforest soils, stream sediments, swamps	This species was first reported by Fernández et al. (2012)	CC, SC	Fernández et al. (2012)
<i>Diffugia lata</i>	Penard, 1942	No	Forest streams, muddy soils, pond sediments, stream sediments	This species was first described by Jung (1942) as <i>Diffugia oblonga lata</i> from samples collected in southern Chile. This species has also been recorded in the northern hemisphere (Beyens and Chardez 1986, 1987)	CC, SC	Jung (1942); Fernández et al. (2012)
<i>Diffugia lebes</i>	Penard, 1883	No	Peatlands	This species was first reported by Zapata (2005)	CC	Zapata (2005); Fernández and Zapata (2011)
<i>Diffugia levantieri</i>	Playfair, 1916	No	Estuaries, forest streams, pond sediments, rainforest mosses, stream sediments, swamps	This species was first reported by Jung (1942)	CC, SC	Jung (1942); Zapata et al. (2002)
<i>Diffugia linearis</i>	Penard 1890	No	Forest streams, peatlands	This species is first reported in the present study	CC	Present study
<i>Diffugia lithophila</i>	Glabronia (<i>Diffugia</i>) <i>lithostictica</i> (Penard) 1902	No	Forest streams	This species was first reported by Jung (1942) under the name of <i>Glabronia (Diffugia) hydrostatica lithophila</i> Jung, 1942	CC, SC	Jung (1942)
<i>Diffugia lobostoma</i>	Leidy, 1876	No	Pond sediments, stream sediments	This species was first reported by Certes (1889)	SC	Certes (1889)
<i>Diffugia lucida</i>	Penard, 1890	No	Lakeshores, lake sediments, on aquatic plants, peatlands, pond sediments, rainforest soils, soil mosses, swamps	This species was first reported by Jung (1942) under the name of <i>Planodiffugia lucida</i> (Penard, 1890)	CC, SC, AN	Jung (1942); Hoogenraad and de Groot (1951); Smith (1978); Zapata and Malamala (1987)
<i>Diffugia oblonga</i>	Ehrenberg, 1838	No	Arid shrublands, humic soil lawns, Mallines, peatlands, pond sediments, rainforest soils, soil mosses, saltmarshes, swamps	This species was first reported by Wallies (1913). Later, Fernández et al. (2012) also first reported the occurrence of <i>Diffugia oblonga curvicolis</i> Varga, 1963	NC, CC, SC, GI	Wallies (1913); Jung (1942); Bonnet (1986); Zapata (2005); Zapata et al. (2007a); Zapata et al. (2008); Fernández and Zapata (2011); Fernández et al. (2012); Fernández (2015)
<i>Diffugia opulenta</i>	Jung, 1942	No	Pond sediments, peatlands, swamps	This species was first described by Jung (1942) as <i>Planodiffugia opulenta</i> Jung, 1942 from samples collected in southern Chile. This species is potentially endemic to central and southern Chile	CC, SC	Jung (1942); Zapata (2005); Fernández and Zapata (2011)
<i>Diffugia penardii</i>	Hopkinson, 1909	No	Lake sediments, on aquatic plants, peatlands, pond sediments, rainforest mosses, soil mosses, swamps	This species was first reported by Jung (1942)	CC, SC	Jung (1942); Zapata (2005); Fernández and Zapata (2011)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Difflugia phida</i>	Ehrenberg, 1842	No	No	Soil mosses	This species was first described by Ehrenberg from soil mosses collected in Cabo de Hornos southern Chile. According to Wales (1913) this species might be considered as a <i>nomini nudum</i> taxon. He argued that the original description of this species is so poor that it is impossible to make a robust conclusion about its taxonomic validity. This author suggested that the individuals observed by Ehrenberg could have corresponded to representatives of the species <i>Cartesia murrayi</i> (Wales, 1913). This species has not been reported since its first description	SC	Ehrenberg (1948)
<i>Difflugia pygmaea</i>	(Jung, 1942)	<i>Eustonia pygmaea</i> Jung, 1942	No	Lakeshores, pond sediments, soils, stream sediments	This species was first described by Jung (1942) as <i>Eustonia pygmaea</i> Jung, 1942 from samples collected in southern Chile. This species is potentially endemic to central and southern Chile	CC, SC	Jung (1942)
<i>Difflugia pyriformis</i>	Perry, 1946	No	<i>D. pyriformis</i> 'cabo de hornos' Certes, 1889	Estuaries, freshwater column, lake sediments, river sediments	This species was first reported by Certes (1889) from samples collected in Cape Horn, Tierra del Fuego Island Chile. Together with the typical form of this species Certes (1889) mentioned the co-occurrence of another version of this taxon which he called as <i>D. pyriformis</i> 'cabo de hornos'. Certes, 1889 because of its occurrence in Cape Horn. According to Certes, the composition of the shell of <i>D. pyriformis</i> 'cabo de hornos' is extremely different from other representatives of the genus <i>Difflugia</i> . Particularly he highlighted the existence of hemispheric structures of diverse size and unknown composition on the shell. These hemispheric structures also were reported by Zapata et al. (2002) in some individuals collected in south-central Chile. <i>D. pyriformis</i> 'cabo de hornos' might be considered as endemic to southern Chile as so far it only has been recorded in this geographical region	CC, SC	Certes (1889); Zapata and Rudolph (1986); Zapata et al. (2002)
<i>Difflugia regularis</i>	(Gauthier-Lormeau and Thomas, 1988)	No	No	Peatlands	This species was first mentioned by Fernández and Zapata (2011)	CC	Fernández and Zapata (2011)
<i>Difflugia sphincta</i>	Jung, 1942	No	No	Lakeshores, swamps	This species was first described by Jung (1942) from samples collected in southern Chile. This species also has been reported in southern Argentina. This species is potentially endemic to southern South America (southern Chile plus southern Argentina) as it only has been recorded in this geographical area	CC, SC	Jung (1942)
<i>Difflugia urceolata</i>	Carter, 1884	<i>Cingodifflugia urceolata</i> (Carter, 1884)	No	Freshwater column, peatlands, pond sediments, stream sediments, swamps	This species was first reported by Certes (1889). Later, Jung (1942) reported this species as <i>Cingodifflugia urceolata</i> (Carter, 1884)	CC, SC	Certes (1889); Jung (1942); Zapata and Rudolph (1986)
<i>Difflugia vancouveri</i>	Boltovskoy and Lena, 1971	No	No	Estuaries, peatlands, river sediments	This species was first reported by Zapata and Rudolph (1986). This species was first described by Boltovskoy and Lena (1971) from samples collected in southern Argentina. This species is potentially endemic to southern South America (southern Chile plus southern Argentina) as it only has been recorded in this geographical area	CC	Zapata and Rudolph (1986); Zapata et al. (2002); Zapata (2005); Fernández and Zapata (2011)
<i>Difflugia venusta</i>	(Perry, 1942)	No	No	Pond sediments	This species is first reported in the present study	CC	Present study
<i>Difflugia</i> sp. 1		No	No	Muddy soils	This taxon was reported by Jung (1942) from muddy soil samples collected in southern Chile	SC	Jung (1942)
<i>Difflugia</i> sp. 2		No	No	Forest streams	This taxon was reported by Jung (1942) from forest stream samples collected in southern Chile	SC	Jung (1942)
<i>Difflugia</i> sp. 3		No	No	Volcanic crater lakes	This taxon was first reported by Zapata and Crespo (1990) from sediment samples collected in a volcanic crater lake, Easter Island	EI	Zapata and Crespo (1990)
Lagenodifflugia	Medell and Sook, 1983						
<i>Lagenodifflugia bryophila</i>	(Perrard, 1902)	<i>Perrardius bryophilus</i> Perrard, 1902; <i>P. bryophila</i> elacrys Jung, 1942	<i>L. bryophila</i> elacrys (Jung, 1942)	Acid mixed soils, humic soil lawns, peatlands, pond sediments, rainforest mosses, soil mosses, <i>Sphagnum</i> mosses, stream sediments	This species was first reported by Jung (1942). This author also first described and reported the co-occurrence of a subtaxa of this species which he called <i>Perrardius bryophila</i> elacrys Jung, 1942	CC, SC	Jung (1942); Bonnet (1966); Zapata et al. (2008)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Lagenodiffugia vas</i>	(Lamy, 1974)	No	No	Lake sediments, peatlands, sediments, saltmarshes	This species was first reported by Certes (1889)	CC, SC	Certes (1889); Zapata (2005); Zapata et al. (2005); Fernández and Zapata (2011)
<i>Lagenodiffugia</i> sp.		No	No	Swamps	This taxon was first described by Fernández et al. (2012)	GI	Fernández et al. (2012)
Pontigulasia	Rhumbler, 1896	No	No	Mosses on lakeshores, peatlands, swamps	This species was first described by Jung (1942) from samples collected in southern Chile. This species is potentially endemic to central and southern Chile	CC, SC	Jung (1942); Zapata (2005); Fernández and Zapata (2011)
<i>Pontigulasia compressoides</i>	Jung, 1942	No	No	Peatlands, pond sediments, swamps	This species was first described by Jung (1942) from samples collected in southern Chile. <i>P. contusa</i> has also been reported in southern Argentina (Vucelich and Lopretto, 1995) as <i>Lagerodiffugia contusa</i> . This species is potentially endemic to southern South America (southern Chile plus southern Argentina) as it only has been recorded in this geographical area	CC, SC	Jung (1942); Zapata (2005); Zapata et al. (2008); Fernández and Zapata (2011)
<i>Pontigulasia contusa</i>	Jung, 1942	No	No				
<i>Pontigulasia rhumbleri</i>	Hopkinson, 1919	No	No	Forest streams	This species was first reported by Jung (1942)	CC, SC	Jung (1942)
Schwabia	Jung, 1942	No	No	Forest streams, lakeshore peatlands, pond sediments, rainforest soils, swamps	The genus <i>Schwabia</i> and this species were first described by Jung (1942) from samples collected in southern Chile. This species has also been reported in the northern hemisphere (Török 1998)	CC, SC	Jung (1942)
<i>Schwabia regularis</i>	Jung, 1942	No	No				
<i>Schwabia terricola</i>	Bonnet and Thomas, 1955	No	No	Rainforest soils, soil mosses	This species was first reported by Bonnet (1966)	CC, SC	Bonnet (1966)
Zivkovicia	Ogden, 1987	No	No	Estuaries, forest streams, river sediments, swamps	This species was first reported by Zapata and Rudolph (1986) under the name of <i>Pontigulasia compressa</i> (Carter, 1864)	CC, SC, GI	Zapata and Rudolph (1986); Zapata et al. (2002); Fernández et al. (2012)
<i>Zivkovicia compressa</i>	(Carter, 1864)	<i>Calligula compressa</i> Carter, 1864; <i>Pontigulasia compressa</i> (Carter, 1864)	No				
.... Hyalospheniidae	Schultz, 1977						
Alcocdera	Jung, 1942	No	No	Forest streams, mosses on peatlands, pond sediments, rainforest soils, soil humus, soil mosses	This species was first reported by Walles (1913) under the name of <i>Nébela cockaynei</i> Penard, 1910	CC, SC	Walles (1913); Jung (1942); Bonnet (1966); Zapata (2005); Zapata et al. (2008); Fernández and Zapata (2011)
<i>Alcocdera cockaynei</i>	(Penard, 1910)	<i>Apodera cockaynei</i> (Penard, 1910); <i>Nébela cockaynei</i> (Penard, 1910)	No				
Apodera	Lusich and Tarjan, 1961	No	No	<i>Sphagnum</i> mosses	This species was first reported by Jung (1942) from samples collected in southern Chile. This species is potentially endemic to south-central Chile	CC, SC	Jung (1942)
<i>Apodera crenata</i>	Jung, 1942	No	No				

Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Acodera vas</i>	<i>Medusa vas</i> Cortes, 1939	<i>A. vas</i> 'a' Zapata et al., 2007a; <i>A. vas</i> 'b' Zapata et al., 2007a; <i>A. vas nicticollis</i> Jung, 1942	Forest streams, humus in trunks, humus on trunks, lakeshores, Maguelan rainforest soils, Mallines, mosses on trunks, muddy soils, North Patagonian rainforest soils, on aquatic plants, peatlands, pond sediments, rainforest soils, river bottoms, saltmarshes, soil humus, soil mosses, swamps	This species was first described by Cortes (1939) under the name of <i>Nebela vas</i> Cortes, 1939 from samples collected in Tierra del Fuego Island, Chile. As this species has never been recorded in the Holartic, it is frequently used to illustrate that at least some eukaryotic free-living microorganisms exhibit restricted geographical distributions. Later, Jung (1942), first described the subspecific taxon <i>A. vas nicticollis</i> Jung, 1942. Several years later Zapata et al. (2007a) reported the occurrence of other two intraspecific taxa which they called <i>A. vas</i> 'a' and <i>A. vas</i> 'b'. These three intraspecific taxa might be considered as being unique to southern Chile (endemics) as they only have been recorded in this geographical region. Moreover, a detailed morphological and morphometric study revealed that shell dimensions and morphology of <i>A. vas sensu lato</i> , exhibit a high variation within and between different populations from southern Chile, suggesting the existence of several pseudocryptic species and thus, maybe also cryptic species, within this taxon (Zapata and Fernández 2008). However, this hypothesis still needs to be tested using extra information (e.g. molecular data)	CC, SC, GL, AN	Cortes (1939); Wallies (1913); Jung (1942); Hogenraad and de Groot (1951); Bonnet (1966); Zapata and Rudolph (1966); Zapata and Malamala (1987); Zapata (2005); Zapata et al. (2007a); Zapata and Fernández (2008); Fernández and Zapata (2011); Fernández et al. (2012)
<i>Argynnia</i>						
<i>Argynnia caudata</i>	<i>Argynnia</i> (1909) (1975)	Ma	Peatlands, rainforest mosses	This species is first reported in the present study	CC, SC	Present study
<i>Argynnia dentistoma</i>	<i>Argynnia dentistoma</i> (Pocock 1907) (Pocock 1907)	Ma <i>Argynnia dentistoma</i> (Pocock 1907) (Pocock 1907)	Forest streams, humic soil lawns, lakeshore mosses, muddy soils, North Patagonian rainforest soils, on aquatic plants, peatlands, pond sediments, rainforest mosses, rainforest soils, soil mosses, stream sediments, swamps	This species was first reported by Wallies (1913)	CC, SC, GL	Wallies (1913); Jung (1942); Bonnet (1966); Zapata (2005); Zapata et al. (2008); Fernández and Zapata (2011); Fernández et al. (2012)
<i>Argynnia gerrudeana</i>	Jung, 1942	Ma	Peatlands, pond sediments, rainforest soils, river sediments, soil mosses, swamps	This species was first described by Jung (1942) from samples collected in southern Chile. <i>A. gerrudeana</i> has been also recorded in southern Argentina (Vucelich and Lopretto 1995). This species is potentially endemic to southern South America (southern Argentina) as this species only has been recorded in this geographical area	CC, SC	Jung (1942); Zapata et al. (2008)
<i>Argynnia schwabei</i>	Jung, 1942	Ma	Forest streams, peatlands, rainforest soils, soil mosses	This species was first described by Jung (1942) from samples collected in southern Chile. <i>A. schwabei</i> Jung, 1942 has been also recorded in southern Argentina as <i>Nebela schwabei</i> (Vucelich and Lopretto 1995). This species is potentially endemic to southern South America (southern Chile plus southern Argentina) as it only has been recorded in this geographical area	CC, SC	Jung (1942)
<i>Argynnia similis</i>	(Vucelich, 1973)	Ma	Peatlands	This species was first described by Vucelich (1973) as <i>Nebela similis</i> Vucelich, 1973 from samples collected in southern Argentina. However, this species undoubtedly belongs to the genus <i>Argynnia</i> (see original description). Here, we report the first occurrence of this species in Chile as <i>A. similis</i> (Vucelich, 1973) comb. nov. This species is potentially endemic to southern South America (southern Chile plus southern Argentina) as this species only has been recorded in this geographical area	SC	Present study
<i>Argynnia teres</i>	Jung, 1942	Ma	Lakeshore mosses, saltmarshes, stream sediments	This species was first described by Jung (1942) from samples collected in southern Chile. <i>A. teres</i> has been also recorded in southern Argentina under the name of <i>Nebela teres</i> (Vucelich and Lopretto 1995). This species is potentially endemic to southern South America (southern Chile and southern Argentina)	CC, SC	Jung (1942)
<i>Argynnia tylophora</i>	Jung, 1942	Ma	Forest streams, rainforest soils	This species was first described by Jung (1942) from samples collected in southern Chile. <i>A. tylophora</i> has been also recorded in southern Argentina under the name of <i>Nebela tylophora</i> (Vucelich and Lopretto 1995). This species is potentially endemic to southern South America (southern Chile and southern Argentina)	CC, SC	Jung (1942)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Argyria vitrea</i>	Perard, 1899	<i>Nelobia vitrea</i> (Perard, 1899), <i>N. vitrea</i> (misapplied synonym to <i>N. vitrea</i>)	<i>A. vitrea minor</i> Wales, 1912	Forest streams, Mallines, muddy soils, North Patagonian rainforest peatlands, pond sediments, stream sediments, swamps	This species was first reported by Jung (1942) together with the subspecific taxon <i>A. vitrea minor</i> Wales, 1912	CC, SC, GI	Zapata (2005), Zapata et al. (2007a); Zapata et al. (2008); Fernández and Zapata (2011); Fernández et al. (2012)
<i>Argyria</i> sp.		No	No	Arid shrublands	This taxon was first described by Fernández (2015)	NC	Fernández (2015)
Certesella	Loeblich and Tappan, 1961						
<i>Certesella australis</i>	(Vucelich, 1973)	No	No	Peatlands, soil mosses	This species is first reported in this study. This species was first described by Vucelich (1974) from samples collected in southern Argentina. This species has also been repeatedly recorded in southern Chile. This species is potentially endemic to southern South America (southern Chile and southern Argentina)	SC	Present study
<i>Certesella certes</i>	(Perard, 1911)	<i>Perardella certes</i> (Perard, 1911), <i>Nelobia certes</i> Perard, 1911	No	Mallines, peatlands, pond sediments, rainforest soils, soil mosses, swamps	This species was first reported by Jung (1942)	CC, SC, AN	Jung (1942); Smith (1978); Zapata (2005); Fernández and Zapata (2011)
<i>Certesella martali</i>	(Cartes, 1899)	<i>Nelobia martali</i> Certes, 1899, <i>Perardella cartesi</i> (Cartes, 1901)	<i>C. martali</i> 'a' Zapata et al., 2008; <i>C. martali</i> 'b' Zapata et al., 2008	Conifer forest soils, forest streams, Mallines, mosses on trunks, muddy soils, North Patagonian rainforest soils, on aquatic plants, peatlands, pond sediments, humus, soil mosses, rainforest soils, soil mosses, <i>Sphagnum</i> mosses, stream sediments, swamps	This species was first described by Certes (1899) from samples collected in Cabo de Hornos, Tierra del Fuego Archipelago, Chile. This species exhibits a high morphological variation. Therefore, various researchers have raised the hypothesis that this taxon represents a species complex. An example of this idea can be found in the manuscript of Zapata et al. (2008). In a clear effort to avoid the lumping of distinctly different morphotypes of this species in only one taxon, these authors decided to divide the representatives they found in <i>C. martali</i> 'a' and <i>C. martali</i> 'b'	CC, SC, GI	Certes (1899); Jung (1942); Bonnet (1966); Zapata and Rudolph (1986); Zapata (2005); Zapata et al. (2007a); Zapata et al. (2008); Fernández and Zapata (2011); Fernández et al. (2012)
<i>Certesella murrayi</i>	(Wales, 1913)	<i>Hypnum proprium</i> Wales, 1913	No	Soil mosses, stream sediments	This species was first described by Wales as <i>Nelobia murrayi</i> from samples collected in Punta Arenas, Chile and Rio de Janeiro, Brazil. Despite this species has a very conspicuous morphology, fact that for this species reduces the possibility of being overlooked, it has not been recorded since its first description in either Chile or Brazil (present study; see also Lansac-Toha 2001 for Brazil)	SC	Wales (1913)
Heleopera							
<i>Heleopera amplipolia</i>	Leidy, 1876 Jung, 1942	No	No	Peatlands, pond sediments, stream sediments	This species was first described by Jung (1942) from samples collected in south-central Chile. This species is potentially endemic to south-central Chile	CC	Jung (1942); Zapata (2005); Fernández and Zapata (2011)
<i>Heleopera petricola</i>	Leidy, 1876	No	<i>H. petricola major</i> Cash, 1909; <i>H. petricola helvola</i> Jung, 1942; <i>H. petricola</i> 'a' Zapata et al., 2008; <i>H. petricola</i> 'b' Zapata et al., 2008	Forest streams, humus in trunks, Mallines, North Patagonian rainforest soils, on aquatic plants, on freshwater algae, peatlands, pond sediments, rainforest soils, soil mosses, <i>Sphagnum</i> mosses, stream sediments, swamps	This species was first reported by Jung (1942) together with <i>H. petricola helvola</i> Cash, 1909 and <i>H. petricola major</i> Jung, 1942. The latter was first described by Jung (1942), from samples collected in southern Chile	CC, SC, GI	Jung (1942); Hoogenraad and de Groot (1951); Bonnet (1966); Zapata (2005); Zapata et al. (2007a); Zapata et al. (2008); Fernández and Zapata (2011); Fernández et al. (2012)
<i>Heleopera reclangulans</i>	Bornet, 1886	No	No	Rainforest soils, peatlands	This species was first described by Bonnet (1886) from rainforest soil samples collected in southern Chile. Within the original description of this species, Bonnet (1886) stated that this species has also been recorded in equatorial Africa. This species has also been reported in the northern hemisphere (Bonnet and Gómez-Sánchez 1994)	CC	Bonnet (1886); Zapata (2005); Fernández and Zapata (2011)
<i>Heleopera rosea</i>	Perard, 1899	No	No	Forest streams, humic soil lawns, mosses on trunks, on freshwater algae, peatlands, swamps	This species was first reported by Jung (1942)	CC, SC	Jung (1942); Zapata et al. (2008)
<i>Heleopera sphagni</i>	Leidy, 1876	<i>Halimoceros pulchra</i> Leidy, 1876	No	Forest streams, North Patagonian rainforest soils, peatlands, pond sediments, stream sediments	This species was first reported by Certes (1889) as <i>H. picta</i> Leidy, 1879 a junior synonym of <i>H. sphagni</i> Leidy, 1874	CC, SC	Certes (1889); Zapata et al. (2005); Fernández and Zapata (2011); Fernández et al. (2012)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Heleopera sylvatica</i>	Peters, 1890	<i>Heleopera sylvatica</i> (L.) <i>muscipallidus</i> (L.) (<i>sylvatica</i>)	No	Lakeshore mosses, on aquatic plants, peatlands, pond sediments, rainforest mosses, rainforest soils, rivers sediments, soil mosses, swamps	This species was first reported by Jung (1942)	CC, SC	Jung (1942); Bonnet (1966); Zapata and Rudolph (1986)
<i>Heleopera</i> sp. 1		No	No	Soil mosses	This taxon was reported by Jung (1942) from soil mosses collected in Calbuco Island, south-central Chile	CC	Jung (1942)
<i>Heleopera</i> sp. 2		No	No	Soil mosses	This taxon was reported by Hoogenraad and de Groot (1951) from soil moss samples (<i>Campylopus inflexus</i> [Hedw.]) collected in south-central Chile	CC	Hoogenraad and de Groot (1951)
<i>Heleopera</i> sp. 3		No	No	Soil mosses	This taxon was reported by Hoogenraad and de Groot (1951) from soil moss samples (<i>Eriodon conostomus</i> Mont.) collected in south-central Chile	CC	Hoogenraad and de Groot (1951)
<i>Heleopera</i> sp. 4		No	No	Peatlands	This taxon was first described by Zapata et al. (2008) from samples collected in south-central Chile	CC	Zapata et al. (2008)
<i>Hyalosphenia</i>							
<i>Hyalosphenia cuneata</i>	(Stein, 1857) Stein, 1857	No	No	Freshwater column, pond sediments, stream sediments	This species was first reported by Certes (1889)	SC	Certes (1889)
<i>Hyalosphenia elegans</i>	Leidy, 1879	No	No	Pond sediments, rainforest soils, soil mosses, stream sediments	This species was first reported by Certes (1889)	CC, SC	Certes (1889); Jung (1942)
<i>Hyalosphenia schoutedeni</i>	Van Oye, 1928	No	No	On aquatic plants, river sediments	This species was first reported by Zapata and Rudolph (1986)	CC, SC	Zapata and Rudolph (1986)
<i>Hyalosphenia subflava</i>	Csiki, 1900	No	No	Conifer forest soils, humus on trunks, lakeshore mosses, muddy soils, peatlands, pond sediments, rainforest soils, humus, soil mosses, stream sediments, swamps	This species was first reported by Wailes (1913)	CC, SC	Wailes (1913); Jung (1942); Bonnet (1966); Zapata et al. (2008); Fernández and Zapata (2011)
<i>Nebela</i>							
<i>Nebela barbata</i>	Leidy, 1879 Leidy, 1879	No	<i>N. barbata psilonota</i> (Jung, 1942)	Forest streams, Mallines, muddy soils, North Patagonian rainforest soils, peatlands, pond sediments, stream sediments, swamps	This species was first reported by Jung (1942) as <i>N. barbata psilonota</i> Jung, 1942. It is noteworthy that so far the typical form of this species has never been recorded in Chile. Only the <i>N. barbata psilonota</i> has been repeatedly recorded in Chile. This fact makes one suspect that <i>N. barbata psilonota</i> could actually be a species and not simple a subspecific taxon. This taxon could be potentially endemic to southern Chile	CC, SC, CI	Jung (1942); Zapata (2005); Zapata et al. (2007a); Zapata et al. (2008); Fernández and Zapata (2011); Fernández et al. (2012)
<i>Nebela bigibbosa</i>	Peters, 1890	No	No	Soil humus	This species was first described by Bonnet (1966)	CC	Bonnet (1966)
<i>Nebela carnulata</i>	Jung, 1942	<i>Pterygia carnulata</i> Jung, 1942	No	Lakeshores	This species was first described by Jung (1942) as <i>Pterygia carnulata</i> Jung, 1942 from samples collected in southern Chile. This species is potentially endemic to south-central Chile	CC, SC	Jung (1942)
<i>Nebela collaris</i>	(Ehrenberg, 1848)	<i>N. helmanilla</i> Taranek, 1882	<i>N. collaris</i> 'a' Certes, 1889; <i>N. collaris</i> 'b' Certes, 1889	Forest streams, freshwater columns, lakeshores, North Patagonian rainforest soils, peatlands, rainforest soils, river sediments, soil mosses, swamps	This species was first reported by Certes (1889) from samples collected in Cape Horn, Tierra del Fuego Island, Chile. Together with the typical form of this species, Certes mentioned the co-occurrence of two extra versions of this taxon which he called informally as <i>N. collaris</i> 'a' and <i>N. collaris</i> 'b'. Jung (1942) and Bonnet (1966) also reported <i>N. bohemica</i> Taranek, 1882. This taxa is currently considered a synonym of <i>N. collaris</i> (Ehrenberg, 1848) (see Kosakyan et al. 2012)	CC, SC, CI, AN	Certes (1889); Wailes (1913); Jung (1942); Bonnet (1966); Zapata and Matamala (1987); Zapata (2005); Zapata et al. (2008); Fernández and Zapata (2011); Fernández et al. (2012)
<i>Nebela fabrei</i>	Certes, 1889	No	No	Pond sediments, stream sediments	This species was first described by Certes (1889) from samples collected in Cabo de Hornos, Tierra del Fuego Archipelago, Chile. It is noteworthy that Certes (1889) warned that this taxon could be a teratological specimen of another species of <i>Nebela</i> . Later, Declaire (1956) reported this species from Madagascar and supported the validity of this species. It is likely that this species exhibits a gondwanic distribution	SC	Certes (1889)
<i>Nebela galeata</i>	Peters, 1902	<i>Umbonaria galeata</i> (Peters, 1902)	No	Lakeshores, stream sediments, soil mosses	This species was first reported by Jung (1942) as <i>Umbonaria galeata</i> Jung, 1942	CC, SC	Jung (1942)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Nebela gracilis</i>	Penard, 1910	<i>Umbonaria gracilis</i> Jung, 1942	No	Malines, swamps	This species was first reported by Jung (1942) as <i>Umbonaria gracilis</i> Jung, 1942	CC, SC	Jung (1942)
<i>Nebela longicollis</i>	Penard, 1900	No	No	Malines	This species was first reported by Zapata et al. (2007a)	CC	Zapata et al. (2007a)
<i>Nebela militaris</i>	Penard, 1900	No	No	Forest streams, lakeshore mosses, pond sediments, rainforest mosses, soil mosses	This species was first reported by Jung (1942)	CC, SC	Jung (1942)
<i>Nebela penardiana</i>	Dallandae, 1936	No	No	Forest streams, humid soil lawns, Malines, North Patagonian rainforest soils, peatlands, pond sediments, rainforest mosses, <i>Sphagnum</i> mosses, swamps	This species was first reported by Jung (1942)	CC, SC, GI	Jung (1942); Bonnet (1966); Zapata (2005); Zapata et al. (2007a); Zapata et al. (2008); Fernández and Zapata (2011); Fernández et al. (2012)
<i>Nebela repanda</i>	(Jung, 1942)	<i>Dallandaea repanda</i> Jung, 1942	No	Forest streams, pond sediments, <i>Sphagnum</i> mosses, swamps	This species was first described by Jung (1942) as <i>Dallandaea repanda</i> Jung, 1942 from samples collected in southern Chile. This species is potentially endemic to south-central Chile	CC, SC	Jung (1942)
<i>Nebela spesirosa</i>	Dallandae, 1936	<i>N. spesirosa</i> (a misspelled name of <i>N. spesirosa</i>)	No	Peatlands	This species was first reported by Zapata et al. (2008) as <i>N. spesirosa</i> (a misspelled name of <i>N. spesirosa</i>). So far it was thought that the geographical distribution of this species was restricted to the Holarctic region. However, while the individual reported by Zapata et al. (2008, Fig. 41) fits very well both the outline and size given in the original description of this species, it is impossible to confirm the validity of this record. The picture provided by Zapata et al. (2008) does not allow the observation of additional morphological traits that characterize this species such as the existence of a slightly concave pseudostoma (in lateral view) and the two lateral pores. This situation suggests that this record still needs to be confirmed by further studies. According to Ogden (1984) <i>N. tubulosa</i> Penard, 1890 can be confused with <i>N. spesirosa</i>	CC	Zapata et al. (2008)
<i>Nebela tincta</i>	(Ledy, 1879)	<i>Hyalosphenia tincta</i> Ledy, 1879; <i>N. tincta</i> Penard, 1902; <i>N. tincta</i> Penard, 1902; <i>N. tincta</i> Ledy, 1879	No	Forest streams, peatlands, pond sediments, rainforest soils	This species was first reported by Certes (1889) as <i>Hyalosphenia tincta</i> Ledy, 1879. Later, Jung (1942) and Bonnet (1966) also reported <i>N. tincta</i> Ledy, 1879 and <i>N. tincta</i> Penard, 1902, respectively. These three taxa are currently considered synonyms of <i>N. tincta</i> Ledy, 1879	CC, SC, AN	Certes (1889); Jung (1942); Bonnet (1966); Smith (1978)
<i>Nebela tubulosa</i>	Penard, 1900	No	No	Humus on trunks	This species was first reported by Bonnet (1966)	CC, SC	Bonnet (1966)
<i>Nebela</i> sp. 1		No	No	Lakeshore	This taxon was reported by Jung (1942) from lakeshore samples collected in southern Chile	SC	Jung (1942)
<i>Nebela</i> sp. 2		No	No	Forest streams, swamps	This taxon was first described by Fernández et al. (2012)	GI	Fernández et al. (2012)
<i>Nebela</i> sp. 3		No	No	Swamps	This taxon was first described by Fernández et al. (2012)	GI	Fernández et al. (2012)
<i>Padaungella</i>	Lara and Todorov, 2012						
<i>Padaungella cordiformis</i>	(Heins, 1914)	<i>Nebela lapidiformis</i> Penard, 1914	No	Humus on trunks, peatlands	This species was first reported by Bonnet (1966) as <i>Nebela lapidiformis cordiformis</i> . Although this name has been used for many years, we think that this species undoubtedly belongs to the genus <i>Padaungella</i> Lara and Todorov, 2012 (see Kosakyan et al. 2012). Thus, we report this species here as <i>P. cordiformis</i> (Heins, 1914) comb. nov.	CC, SC	Bonnet (1966); Zapata et al. (2008)
<i>Padaungella lageniformis</i>	(Penard, 1907)	<i>Nebela lageniformis</i> Penard, 1907; <i>Stenanthidium lageniformis</i> Jung, 1942	No	Conifer forest soils, forest streams, humus on trunks, lakeshore, mosses on trees, North Patagonian rainforest soils, peatlands, pond sediments, rainforest soils, soil humus, soil mosses, swamps	This species was first reported by Wailes (1913) as <i>Nebela lageniformis</i> . Although this name has been used for many years, we think that this species undoubtedly belongs to the genus <i>Padaungella</i> Lara and Todorov, 2012 (see Kosakyan et al. 2012). Thus, we report this species here as <i>P. lageniformis</i> (Penard, 1907) comb. nov.	CC, SC, GI, AN	Wailes (1913); Jung (1942); Hoogenraad and de Groot (1951); Bonnet (1966); Zapata and Matamala (1987); Zapata et al. (2008); Fernández et al. (2012)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Padaungella longitubulata</i>	(Gautier-Livré, 1953)	No	No	Forest soils, peatlands	This species is first reported here as <i>Padaungella longitubulata</i> (Gautier-Livré, 1953) comb. nov. Originally, this species was first described as <i>Nebela longitubulata</i> by Gautier-Livré (1953), but because of its morphology, we think that this species certainly belongs to the genus <i>Padaungella</i> Lara and Todorov, 2012 (see Kosakyan et al. 2012). Thus, we report this species here as <i>P. longitubulata</i> (Brown, 1910) comb. nov.	CC, SC	Present study
<i>Padaungella tubulata</i>	(Brown, 1910)	<i>Nebela tubulata</i> , Brown, 1910; <i>Schmidzia tubulata</i> Jung, 1942	<i>P. tubulata</i> 'unnamed form' (Jung, 1942)	Acid mixed soils, forest streams, pond sediments, rainforest soils, soil humus, <i>Sphagnum</i> mosses, swamps	This species was first reported by Jung (1942) as <i>Schmidzia tubulata</i> (Brown, 1910). Later, Bonnet (1966) also reported the occurrence of this species under its original name (<i>Nebela tubulata</i> Brown, 1910). However, while this name has been used for years, we think that this species undoubtedly belongs to the genus <i>Padaungella</i> Lara and Todorov, 2012 (see Kosakyan et al. 2012). Thus, we report this species here as <i>P. tubulata</i> (Brown, 1910) comb. nov.	CC, SC	Jung (1942); Bonnet (1966)
<i>Padaungella wallesi</i>	(Deltorov, 1980)	<i>Nebela wallesi</i> , Deltorov, 1980; <i>Schmidzia wallesi</i> (Deltorov, 1980)	No	Acid mixed soils, pond sediments, humus on trunks, Magellanic rainforest soils, peatlands, rainforest soils, soil humus, soil mosses	This species was first reported by Jung (1942) as <i>Schmidzia wallesi</i> (Brown, 1910). Later, Bonnet (1966) also reported the occurrence of this species under its original name (<i>Nebela tubulata</i> Brown, 1910). However, while this name has been used for years, we think that this species undoubtedly belongs to the genus <i>Padaungella</i> Lara and Todorov, 2012 (see Kosakyan et al. 2012). Thus, we report this species here as <i>P. wallesi</i> (Brown, 1910) comb. nov.	NC, CC, SC	Jung (1942); Bonnet (1966)
<i>Padaungella wetkampii</i>	(Jung, 1942)	<i>Nebela wetkampii</i> (Jung, 1942); <i>Schmidzia wetkampii</i> Jung, 1942	No	Peatlands, pond sediments, <i>Sphagnum</i> mosses, stream sediments	This species was first described by Jung (1942) as <i>Schmidzia wetkampii</i> from samples collected in southern Chile. Later, other authors (Zapata 2005; Fernández and Zapata 2011) also reported this species but as <i>Nebela wetkampii</i> (Jung, 1942). Although this name has been used for years, we think that this species undoubtedly belongs to the genus <i>Padaungella</i> Lara and Todorov, 2012 (see Kosakyan et al. 2012). Thus, we report this species here as <i>P. wetkampii</i> (Jung, 1942) comb. nov. This species is potentially endemic to south-central Chile	CC, SC	Jung (1942); Zapata (2005); Fernández and Zapata (2011)
Physochila							
<i>Physochila corniculata</i>	Jung, 1942	No	No	Rainforest soils	This species was first described by Jung (1942) from samples collected in southern Chile. This species is potentially endemic to southern Chile	CC, SC	Jung (1942)
<i>Physochila griseola</i>	(Pérez, 1991)	No	No	Pond sediments	This species was first reported by Jung (1942)	CC, SC	Jung (1942)
<i>Physochila tenella</i>	(Pojarko, 1993)	No	No	Forest streams, rainforest soils, swamps	This species was first reported by Jung (1942)	CC, SC	Jung (1942)
.... Lesquereusiidae							
Lesquereusia							
<i>Lesquereusia modesta</i>	Schumbeier, 1949	No	No	Malines, peatlands	This species was first reported by Zapata (2005)	CC	Zapata (2005); Zapata et al. (2007a); Fernández and Zapata (2011)
<i>Lesquereusia spiralis</i>	(Ehrhard, 1940)	<i>Lesquereusia spiralis</i> (Ehrhard, 1940); <i>Heblia microscopica</i> (Ehrhard, 1940) (names of the genus <i>Lesquereusia</i>)	<i>L. spiralis</i> 'unnamed form' Jung, 1942	Lakeshores, Malines, peatlands, pond sediments, <i>Sphagnum</i> mosses, stream sediments, swamps	This species was first reported by Jung (1942) as <i>Lecquereusia spiralis</i> (i.e., <i>Lecquereusia</i> is a synonym of the genus <i>Lesquereusia spiralis</i>)	CC, SC	Jung (1942); Zapata et al. (2007a); Zapata et al. (2008)
<i>Lesquereusia</i> sp.		No	No	On aquatic plants, river sediments	This taxon was reported by Zapata and Rudolph (1986) from samples collected in the Damas River, south-central Chile	CC	Zapata and Rudolph (1986)
Netzelia							
<i>Netzelia tuberculata</i>	Ogden, 1979 (Wellisch, 1964)	No	No	<i>Sphagnum</i> mosses, lakeshore sediments	This species is first reported in the present study	CC	Present study
<i>Netzelia wallesi</i>	(Ogden, 1980)	No	No	Arid shrublands	This species is first reported in the present study	NC, CC	Fernández (2015)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
Quadrullella	Cockerell, 1909						
<i>Quadrullella tropica</i>	(Walles, 1912)	<i>Nelstein tropica</i> Walles, 1912	No	Pond sediments, rainforest soils, <i>Sphagnum</i> mosses, stream sediments	This species was first reported by Walles (1913) as <i>Nebela tropica</i> Walles, 1912	SC	Walles (1913)
<i>Quadrullella scutellata</i>	(Walles, 1912)	<i>Nelstein scutellata</i> Walles, 1912	No	Forest streams, soil mosses	This species was first reported by Walles (1913) as <i>Nebela scutellata</i> Walles, 1912	SC	Walles (1913)
<i>Quadrullella symmetrica</i>	(Wallich, 1863)	No	No	Forest streams, on aquatic plants	This species was first reported by Jung (1942)	CC, SC	Jung (1942)
.... Paraquadrulidae							
Paraquadrula	Dellandrie, 1929						
<i>Paraquadrula irregularis</i>	(Archer, 1877)	No	No	Humic soil lawns, rainforest mosses	This species was first reported by Bonnet (1966)	CC	Bonnet (1966)
.... Plagiopyxidae							
Bullinularia	Bonnet and Thomas, 1955						
<i>Bullinularia indica</i>	(Penard, 1911)	<i>Bullinularia indica</i> (Penard, 1911) <i>Bullinularia indica</i> (a misspelled name of <i>B. indica</i>)	No	Estuaries, humus on trunks, Mallines, mosses on trunks, peatlands, river sediments, soil humus, soil mosses	This species was first reported by Hoogenraad and De Groot (1951) as <i>Bullinula indica</i> . Later Zapata and Rudolph (1966) reported this species as <i>Bullinularia indica</i> . In both cases the name was misspelled	CC	Hoogenraad and de Groot (1951); Bonnet (1966); Zapata and Rudolph (1966); Zapata et al. (2002); Zapata (2005); Zapata et al. (2007a); Zapata et al. (2008); Fernández and Zapata (2011)
Plagiopyxis	Penard, 1910						
<i>Plagiopyxis cellula</i>	Penard, 1910	No	No	Forest streams, humus in trunks, rainforest soils, soil mosses, volcanic crater lakes, swamps	This species was first reported by Walles (1913)	CC, SC, EI	Walles (1913)
<i>Plagiopyxis declivis</i>	Bonnet and Thomas, 1955	No	No	Arid shrublands, humus in trunks, humic soil lawns, rainforest soils	This species was first reported by Bonnet (1966)	NC, CC	Bonnet (1966); Fernández (2015)
<i>Plagiopyxis glyptostoma</i>	Bonnet, 1959	No	<i>P. glyptostoma major</i> Bonnet, 1959	Stream sediments, swamps	This species was first reported by Fernández et al. (2012) as <i>P. glyptostoma major</i> Bonnet, 1959	CC, SC, GI	Fernández et al. (2012)
<i>Plagiopyxis labiata</i>	Penard, 1911	No	No	Humus on trunks, soil humus, soil mosses	This species was first reported by Bonnet (1966)	CC	Bonnet (1966)
<i>Plagiopyxis minuta</i>	Bonnet, 1959	No	No	Humus on trunks, rainforest soils	This species was first reported by Bonnet (1966)	CC	Bonnet (1966)
<i>Plagiopyxis oblonga</i>	(Bonnet and Thomas, 1955)	No	No	Humus in trunks, rainforest soils	This species was first reported by Bonnet (1966)	CC	Bonnet (1966)
<i>Plagiopyxis penardi</i>	Thomas, 1958	No	No	Peatlands	This species is first reported in the present study	CC, SC	Present study
<i>Plagiopyxis pusilla</i>	(Bonnet, 1961)	<i>P. callida pusilla</i> Bonnet, 1961	No	Rainforest soils	This species was first reported by Bonnet (1966) as <i>P. callida pusilla</i> Bonnet, 1961	CC	Bonnet (1966)
.... Trigonopyxidae							
Cyclopyxis	Loeblich and Tappan, 1964						
<i>Cyclopyxis ambigua</i>	Dellandrie, 1929	No	No	Estuaries, rainforest soils, river sediments	This species was first reported by Bonnet (1966)	CC	Bonnet (1966); Zapata and Rudolph (1966); Zapata et al. (2007a)
<i>Cyclopyxis arcelloides</i>	(Penard, 1902)	<i>Cyclopyxis arcelloides</i> Penard, 1902	No	Estuaries, forest streams, lake sediments, Mallines, North Palagonian rainforest soils, peatlands, pond sediments, soil mosses, volcanic crater lakes, swamps	This species was first reported by Walles (1913). Later this species was reported as <i>Centropyxis arcelloides</i> Penard, 1902 by Jung (1942). Zapata et al. (2008) and Fernández and Zapata (2011). However, this latter taxon is synonym of <i>Cyclopyxis arcelloides</i> (Penard, 1902)	CC, SC	Walles (1913); Zapata and Crespo (1990); Zapata et al. (2002); Zapata (2005); Zapata et al. (2008); Fernández and Zapata (2011); Fernández et al. (2012)
<i>Cyclopyxis arenata</i>	(Gushman 1930)	No	No	Peatlands	This species was first reported by Zapata (2005)	CC, SC	Zapata (2005); Fernández and Zapata (2011)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Cyclopyxis eurystoma</i>	Deilander, 1929	<i>Centropyxis eurystoma parvula</i> Bonnet and Thomas, 1960	<i>C. eurystoma alta</i> Jung, 1942; <i>C. eurystoma parvula</i> (Bonnet and Thomas, 1960)	Arid shrublands, estuaries, river sediments, humic soil lawns, lake sediments, lakeshores, Magellanic rainforest soils, muddy soils, on aquatic plants, peatlands, pond sediments, rainforest mosses, rainforest soils, soil mosses, <i>Sphagnum</i> mosses, stream sediments, swamps	This species was first reported by Jung (1942) along with the description of a new infraspecific taxon named as <i>C. eurystoma alta</i> Jung, 1942. Then, within the same manuscript, Jung misspelled the name of this species (<i>Centropyxis eurystoma alta</i> , page 259, sample N° 5). Later, Bonnet (1966) reported the co-occurrence of <i>C. eurystoma parvula</i> (Bonnet and Thomas, 1960). <i>C. eurystoma alta</i> Jung, 1942 might be considered as endemic to southern Chile as so far it only has been recorded in this geographical region	NC, CC, SC, GI, AN	Jung (1942); Bonnet (1966); Zapata and Matamala (1987); Zapata et al. (2002); Fernández et al. (2012); Fernández (2015)
<i>Cyclopyxis humilis</i>	Bonnet, 1962	No	No	Arid shrublands, estuaries, river sediments, on aquatic plants, peatlands	This species was first reported by Zapata and Rudolph (1986)	NC, CC	Zapata and Rudolph (1986); Zapata et al. (2002); Zapata (2005); Fernández (2015)
<i>Cyclopyxis intermedia</i>	Kuffner, 1932	<i>Cyclopyxis gigantea</i> Bonnet, 1963	No	Muddy soils, rainforest soils, stream sediments	This species was first reported by Bonnet (1966) as <i>C. gigantea</i> Bonnet, 1963. According to Fossner and Koganova (1995) and Golemansky and Todorov (2000), <i>C. gigantea</i> is a junior synonym of <i>C. intermedia</i> . Here, we accepted the recommendation of these authors	CC, SC, GI	Bonnet (1966); Fernández et al. (2012)
<i>Cyclopyxis kahli</i>	Deilander, 1929	No	No	Arid shrublands, muddy soils, on aquatic plants, peatlands, rainforest soils, sandy supralittoral, stream sediments	This species was first reported by Jung (1942). Then, within the same manuscript, Jung misspelled the name of this species because he mentioned it as <i>Centropyxis kahli</i>	NC, CC, SC, GI	Bonnet (1966); Golemansky and Todorov (1996); Fernández et al. (2012); Fernández (2015)
<i>Cyclopyxis maynei</i>	Kuffner, 1933	No	No	Peatlands	This species is first reported in the present study	CC, SC	Present study
<i>Cyclopyxis penardi</i>	(Penard, 1911)	No	No	River sediments	This species was first reported by Zapata and Rudolph (1986)	CC	Zapata and Rudolph (1986)
<i>Cyclopyxis plana</i>	Bartol, 1963	No	No	Volcanic crater lakes	This species was first reported by Zapata and Crespo (1990)	EI	Zapata and Crespo (1990)
<i>Cyclopyxis stellata</i>	(Wallis, 1927)	<i>Centropyxis stellata</i> Wallis, 1927	No	On aquatic plants, river sediments	This species was first reported by Zapata and Rudolph (1986) as <i>Centropyxis stellata</i>	CC	Zapata and Rudolph (1986)
<i>Cyclopyxis</i> sp. 1		No	No	Forest streams, swamps	This taxon was first described by Fernández et al. (2012)	GI	Fernández et al. (2012)
<i>Cyclopyxis</i> sp. 2		No	No	Arid shrublands	This taxon was first described by Fernández (2015)	NC	Fernández (2015)
<i>Trigonopyxis</i>	Penard, 1912						
<i>Trigonopyxis arcuata</i>	(Lohy, 1879)	<i>Diffugia arcuata</i> Leidy, 1879	No	Estuaries, freshwater column, humus in trunks, humus on trunks, Mallines, peatlands, pond sediments, rainforest soil, rainforest mosses, river sediments, soil humus, soil mosses, swamps	This species was first reported by Certes (1889) as <i>Diffugia arcuata</i> Leidy, 1879	CC, SC	Certes (1889); Jung (1942); Bonnet (1966); Zapata and Rudolph (1986); Zapata et al. (2002); Zapata et al. (2005); Zapata et al. (2007a); Fernández and Zapata (2011)
<i>Trigonopyxis microstoma</i>	Hogenraad and de Groot, 1946	No	No	Peatlands, soil mosses	This species was first reported by Hogenraad and de Groot (1951)	CC, SC	Hogenraad and de Groot (1951); Zapata et al. (2005); Fernández and Zapata (2011)
<i>Trigonopyxis minuta</i>	Schönborn and Friesche, 1988	No	No	Peatlands	This species is first reported in the present study	NC, CC	Present study
... <i>Phryganellina</i>	Bowes, 1985						
.... <i>Cryptodiffugiidae</i>	Jung, 1942						
<i>Cryptodiffugia</i>	Penard, 1900						
<i>Cryptodiffugia larcolata</i>	Golemansky, 1989	No	No	Sandy supralittoral	This species was first reported by Golemansky and Todorov (1996)	SC	Golemansky and Todorov (1996)
<i>Cryptodiffugia oviformis</i>	Penard, 1890	<i>Diffugiella oviformis fusca</i> Penard, 1890	<i>C. oviformis fusca</i> (Penard, 1890)	Arid shrublands, rainforest soils, volcanic crater lakes	This species was first reported by Bonnet (1966) as <i>Diffugiella oviformis fusca</i> Penard, 1890	SC, EI	Bonnet (1966)
<i>Cryptodiffugia sacculus</i>	Penard, 1902	No	No	Lakeshore mosses, pond sediments, stream sediments	This species was first reported by Jung (1942)	CC	Jung (1942)
<i>Cryptodiffugia valida</i>	Playfair, 1917	No	No	Pond sediments, stream sediments	This species was first reported by Jung (1942)	CC	Jung (1942)
<i>Cryptodiffugia vulgaris</i>	(Friesche, 1913)	No	No	Volcanic crater lakes	This species was first reported by Zapata and Crespo (1990)	EI	Zapata and Crespo (1990)
<i>Cryptodiffugia</i> sp.		No	No	Soil mosses	This taxon was reported by Smith (1978) from moss samples collected in the Antarctic	AN	Smith (1978)
.... <i>Phryganellidae</i>	Jung, 1942						

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
Phryganella	Peñard, 1902						
<i>Phryganella acropodia</i>	(Hartig and Lesauv. 1874)	<i>P. hemisphaerica</i> Peñard, 1902; <i>P. hemisphaerica</i> f. <i>hemisphaerica</i> (Hartig)	No	Acid mixed soils, arid shrublands, forest streams, humid soil lawns, humus in trunks, humus on trunks, on freshwater algae, rainforest soils, soil humus, soil mosses	This species was first reported by Wailes (1913) as <i>P. hemisphaerica</i> (a misspelled version of the synonym <i>P. hemisphaerica</i> Peñard, 1902). Then, Hoogenraad and de Groot (1951) also reported this species as <i>P. hemisphaerica</i>	NC, CC, SC, AN	Wailes (1913); Jung (1942); Hoogenraad and de Groot (1951); Bonnet (1966); Smith (1978); Fernández (2015)
<i>Phryganella paradoxa</i>	Peñard, 1902	No	<i>P. paradoxa</i> <i>alia</i> Bonnet and Thomas, 1960	Humus in trunks, humus on trunks, Maguelan rainforest soils, on freshwater algae, rainforest soils	This species was first reported by Jung (1942). Later, Bonnet (1966) reported the occurrence of the infraspecific taxon <i>P. paradoxa alia</i> Bonnet and Thomas, 1960	CC	Jung (1942); Bonnet (1966)
<i>Phryganella</i> sp. 1		No	No	Soil mosses	This taxon was reported by Jung (1942) from soil moss samples collected in southern Chile	SC	Jung (1942)
<i>Phryganella</i> sp. 2		No	No	Arid shrublands	This taxon was first described by Fernández (2015)	NC	Fernández (2015)
Wailesella	Delearden, 1928						
<i>Wailesella eboracensis</i>	(Wailes and Peñard, 1911)	No	No	Peatlands, <i>Sphagnum</i> mosses	This species was first reported by Jung (1942)	CC, SC	Jung (1942)
... Incertae sedis Arcellinida:							
Oopyxis							
<i>Oopyxis cophostoma</i>	Jung, 1942	No	No	Forest streams, peatlands, pond sediments, rainforest mosses, soil mosses, swamps	This genus and species were first described by Jung (1942) from samples collected in southern Chile. This species has also been reported in southern Argentina (Vucelich 1975) and Ecuador (Krashevskaya et al. 2007). This species is potentially endemic to South America	CC, SC	Jung (1942)
... Incertae sedis Arcellinida:							
Paranebela							
<i>Paranebela</i>	Jung, 1942	No	No	Forest streams, lakeshores, muddy soils, rainforest mosses	This genus and species were first described by Jung (1942) from samples collected in southern Chile. While this genus and species are valid, the original description is not good enough to determine exactly what type of organism is <i>P. dentatula</i> . In addition, these taxa have not been reported again since their first mention. These facts cast doubt on the validity of this genus and species. Moreover, if these taxa do exist, they could be potentially endemic to southern Chile. Jung also reported the occurrence of <i>P. dentatula longula</i> Jung, 1942	CC, SC	Jung (1942)
... Incertae sedis Arcellinida:							
Pseudawerintzowia							
<i>Pseudawerintzowia calicula</i>	Bonnet, 1966	No	<i>P. calicula</i> <i>pellit</i> forma Bonnet, 1960	Rainforest soils	This species was first reported by Bonnet (1966)	CC	Bonnet (1966)
.. Himatismenida	Page, 1907						
... Incertae sedis Himatismenida:							
Cochilopodiidae							
.... Cochilopodiidae							
<i>Cochilopodium</i>	de Suedelew, 1934						
	Hartig and Lesauv. 1874						

	Taxonomic authority	Synonym	Subspecific taxa	Habitat	Additional notes	Distribution	References
<i>Coetiliopodium blimbosum</i>	Fuentealba, 1855	No	1/1	Pond, sediments, soil sediments	This species was first reported by Wallès (1913)	SC	Wallès (1913)

SUPPORTING INFORMATION

Table S3 Checklist of SAR taxa recorded in Chile. The classification of the species at higher taxonomic levels follows (except when noticed otherwise) that of Adl et al. (2012). Only the synonyms, subspecific taxa and habitats mentioned in the Chilean literature are listed. The 'Additional notes section' shows extra information for each species. The 'Distribution section' shows the geographical distribution of each species, where the abbreviations indicate: NC (northern continental Chile), CC (central continental Chile), SC (southern continental Chile), EI (Easter Island), GI (Guambin Island), AN (the Chilean Antarctic territory). The 'References section' shows the studies that report each species in Chile.

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
•	Rhizaria						
••	Cercozoa						
•••	Infractea						
••••	Silicoflorea						
•••••	Euglyphida						
••••••	Assulinidae						
	Assulina						
	<i>Assulina collaris</i>	No	No	Peatland	This species is first reported in the present study. The individuals observed in Chile are relatively bigger than the individuals described by Kufferath (1932) from Africa. Length reported by Kufferath: 52 µm (Africa). Total length observed in this study: 83 µm (Chile)	SC	Present study
	<i>Assulina muscorum</i>	<i>A. muscorum</i> (missspelled version of <i>A. muscorum</i>)	No	Glacial rivers, humus in trunks, Mallines, mosses on trees, peatlands, pond sediments, rainforest mosses, rainforest soils, soil humus, soil mosses, stream sediments, swamps	This species was first reported by Wailes (1913)	CC, SC, AN	Wailes (1913); Jung (1942); Hoogenraad and de Groot (1951); Bonnet (1966); Smith (1978); Smith 1985; Zapata et al. (2007a, 2007b); Fernández and Zapata (2011)
	<i>Assulina seminulum</i>	<i>A. seminulum</i> (missspelled version of <i>A. seminulum</i>)	No	Humus on trunks, mosses and lichens covered by snow, peatlands, pond sediments, rainforest soils, soil mosses, stream sediments	This species was first reported by Wailes (1913) as <i>A. seminulum</i> (a misspelled version of <i>A. seminulum</i>)	CC, SC	Curies (1889); Jung (1942); Bonnet (1966); Zapata et al. (2007b); Zapata et al. (2008); Fernández and Zapata (2011)
	Placocista						
	<i>Placocista glabra</i>	No	No	Rainforest soils	This species was first reported by Jung (1942) under the note 'questionable finding'. The occurrence of this species was later confirmed by Bonnet (1966). In addition, this species was found in samples analyzed in the present study	CC, SC	Jung (1942); Bonnet (1966)
	<i>Placocista sphrosa</i>	No	No	Soil mosses, peatlands	This species is first reported in the present study	CC, SC	Present study
	<i>Placocista</i> sp.	No	No	Soil mosses	This taxa was reported by Hoogenraad and de Groot (1951) from moss samples (<i>Campylopus introflexus</i> (Hedw.)) collected in Central Chile	CC	Hoogenraad and de Groot (1951)
•••••	Cyphoderidae						
	Corythionella						
	<i>Corythionella acolia</i>	No	No	Sandy supralittoral	This species was first reported by Golemansky and Todorov (1996)	SC	Golemansky and Todorov (1996)
	<i>Corythionella minima</i>	No	No	Sandy supralittoral	This species was first reported by Golemansky and Todorov (1996)	SC	Golemansky and Todorov (1996)
	Cyphoderia						
	<i>Cyphoderia ampulla</i>	No	No	Humic soil lawns, Mallines, on aquatic plants, peatlands, pond sediments, rainforest soils, river sediments, soil mosses	This species was first reported by Wailes (1913)	CC, SC	Wailes (1913); Jung (1942); Bonnet (1966); Zapata et al. (2007a, 2007b); Zapata et al. (2008); Fernández and Zapata (2011)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Pseudocorythion</i>	Valkarova, 1970						
<i>Pseudocorythion acutum</i>	(Wallies, 1927)	No	No	Sandy supralittoral	This species was first reported by Golemansky and Todorov (1996)	SC	Golemansky and Todorov (1996)
<i>Pseudocorythion wallesi</i>	Golemansky, 1971	No	No	Sandy supralittoral	This species was first reported by Golemansky and Todorov (1996)	CC, SC	Golemansky and Todorov (1996)
..... Euglyphidae	Wallich, 1866a						
<i>Euglypha</i>	Dujardin, 1841						
<i>Euglypha acanthophora</i>	(Ehrenberg, 1841)	No	<i>E. acanthophora subcylindrica</i> Deflandre, 1953; <i>Euglypha acanthophora flexuosa</i> Penard, 1902	Freshwater column, rainforest streams, lakeshores, pond sediments	This species was first reported by Jung (1942) together with the subtaxa <i>E. acanthophora subcylindrica</i> Deflandre, 1953 and <i>E. acanthophora flexuosa</i> Penard, 1902	CC, SC	Jung (1942); Zapata and Rudolph (1986)
<i>Euglypha alveolata</i>	Ledy, 1879	No	No	Pond sediments, rainforest streams, soil mosses	This species was first reported by Certes (1889)	NC, CC, SC	Certes (1889); Walles (1913)
<i>Euglypha castrii</i>	Bonnét, 1966	No	No	Humus on trunks, Maguelian forest soils, rainforest soils	This species was first described by Bonnét (1966) from samples collected in southern Chile. This species has also been reported in French Guyana (Couteaux 1979) and Mexico (Bonnét 1977). It is likely that this species exhibits a neotropical geographical distribution	CC	Bonnét (1966)
<i>Euglypha ciliata</i>	(Ehrenberg, 1848)	No	No	Freshwater column, humus on trunks, mosses and lichens covered by snow, mosses on trees, peatlands, pond sediments, rainforest soils, soil mosses, <i>Sphagnum</i> mosses	This species was first reported by Certes (1889)	CC, SC	Certes (1889); Walles (1913); Jung (1942); Zapata (2005); Zapata et al. (2007b); Fernández and Zapata (2011)
<i>Euglypha compressa</i>	Carter, 1880	No	No	Rainforest streams, rainforest soils, soil mosses	This species was first reported by Walles (1913)	CC	Walles (1913); Bonnét (1966); Zapata et al. (2007b)
<i>Euglypha cristata</i>	Ledy, 1879	No	<i>E. cristata decora</i> Jung, 1942; <i>E. cristata acicularis</i> Walles, 1912	Arid shrublands, humic soil lawns, lake sediments, lakeshores, muddy soils, on aquatic plants, pond bottoms, rainforest mosses, soil mosses, <i>Sphagnum</i> mosses, swamps	This species was first reported by Walles (1913). Along with the typical form, Jung (1942) reported the occurrence of <i>E. cristata decora</i> Jung, 1942. Later, Fernández (2015) reported the occurrence of <i>E. cristata acicularis</i> Walles, 1912 in and soils of northern Chile	NC, CC, SC, AN	Walles (1913); Jung (1942); Bonnét (1966); Zapata and Matamala (1987); Fernández and Zapata (2011); Fernández (2015)
<i>Euglypha cuspidata</i>	Bonnét, 1969	No	<i>E. cuspidata</i> 'petite forme' Bonnét, 1966	Humus in trunks, mosses on trunks, rainforest soils	This species was first reported by Bonnét (1966) together with the variety <i>E. cuspidata</i> forme 'petite Bonnét, 1966'. The infraspecific taxon might be considered as endemic to southern Chile as so far it only has been recorded in this geographical region	CC	Bonnét (1966)
<i>Euglypha denticulata</i>	Brown, 1912	No	No	Rainforest streams, rainforest soils, soil mosses	This species was first reported by Walles (1913)	NC, CC, SC	Walles (1913); Bonnét (1966)
<i>Euglypha dolioformis</i>	Bonnét, 1969	No	No	Rainforest soils	This species was first reported by Bonnét (1966)	CC	Bonnét (1966)
<i>Euglypha fillera</i>	Penard, 1900	No	No	Conifer forest soils, lake sediments, lakeshores, mosses and lichens covered by snow, peatlands, pond sediments, rainforest soils, <i>Sphagnum</i> mosses, swamps	This species was first reported by Jung (1942)	CC, SC	Jung (1942); Bonnét (1966); Zapata (2005); Zapata et al. (2007b); Zapata et al. (2008); Fernández and Zapata (2011)
<i>Euglypha laevis</i>	(Ehrenberg, 1832)	No	No	Acid mixed soils, hot springs, humic soil lawns, humus in trunks, lake sediments, lakeshores, mosses on trees, muddy soils, on freshwater algae, on aquatic plants, peatlands, pond sediments, rainforest soils, <i>Sphagnum</i> mosses, swamps, volcanic crater lakes	This species was first reported by Walles (1913)	CC, SC; EI, AN	Walles (1913); Jung (1942); Hoogenraad and de Groot (1951); Bonnét (1966); Smith (1978); Zapata and Matamala (1987); Zapata and Crespo (1990); Zapata (2005); Fernández and Zapata (2011)
<i>Euglypha mucronata</i>	Ledy 1878	No	No	Lakeshores, peatlands, pond bottoms	This species was first reported by Certes (1889)	CC, SC	Certes (1889); Zapata et al. (2005); Fernández and Zapata (2011)
<i>Euglypha polylepis</i>	Bonnét, 1969	No	No	Humus in trunks, rainforest soils	This species was first reported by Bonnét (1966)	CC	Bonnét (1966)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
<i>Euglypha recurvispina</i>	Bonnet, 1966	No	No	Humus on trunks, mosses and lichens covered by snow	This species was first described by Bonnet (1966) from samples collected in southern Chile. This species has also been recorded in Ecuador (Krashevskaya et al. 2007). This species might be potentially considered as endemic to South America as so far it only has been recorded in two South American countries	CC	Bonnet (1966); Zapata et al. (2007b)
<i>Euglypha rotunda</i>	Waller and Penard, 1911	No	<i>E. rotunda curvata</i> Jung, 1942; <i>E. rotunda minor</i> Wallies, 1913; <i>E. rotunda toxodora</i> Jung, 1942	Acid mixed soils, humid soil lawns, humus in trunks, lakeshores, mosses on trunks, rainforest soils, <i>Sphagnum</i> mosses	This species was first reported by Jung (1942) as <i>E. rotunda curvata</i> Jung, 1942 and <i>E. rotunda toxodora</i> Jung, 1942. Later, Bonnet (1966) reported this species as <i>E. rotunda minor</i> Wallies, 1913. The typical form was first reported by Zapata and Matamala (1987)	CC, SC, AN	Jung (1942); Bonnet (1966); Smith (1978); Smith 1985; Zapata and Matamala (1987)
<i>Euglypha strigosa</i>	Elveberg, 1871	No	<i>E. strigosa glabra</i> Wallies, 1938	Acid mixed soils, arid shrublands, forest streams, glacial rivers, humid soil lawns, on freshwater algae, Mallines, mosses and lichens covered by snow, mosses on trunks, peatlands, pond bottoms, rainforest soils, soil mosses, swamps	This species was first reported by Wallies (1913). Wallies (1913), Bonnet (1966) and Jung (1942) reported the occurrence of <i>E. strigosa glabra</i> Wallies, 1938 together with the typical form. Meisterfeld (2009) mentioned that members of <i>Euglypha</i> frequently break off in empty shells and then possibly lead to confusion with the so-called 'glabra-forms'	NC, CC, SC, AN	Wallies (1913); Jung (1942); Bonnet (1966); Smith (1978); Smith 1985; Zapata (2005); Zapata et al. (2007a, 2007b); Zapata et al. (2008); Fernández and Zapata (2011); Fernández (2015)
<i>Euglypha tuberculata</i>	DuRoi, 1841	No	<i>E. tuberculata minor</i> Taranek, 1882; <i>E. tuberculata cirrata</i> Wallies (year?)	Forest streams, freshwater column, humid soil lawns, lakeshores, Mallines, on aquatic plants, peatlands, pond bottoms, rainforest soils, soil mosses, <i>Sphagnum</i> mosses, swamps, volcanic crater lakes	This species was first reported by Jung (1942). Bonnet (1966) reported the occurrence of <i>E. tuberculata minor</i> Taranek, 1882. Later, Zapata and Rudolph (1986) reported <i>E. tuberculata cirrata</i> Wallies (year?) and <i>E. tuberculata minor</i> Taranek, 1882	NC, CC, SC, EI, AN	Jung (1942); Bonnet (1966); Zapata and Matamala (1987); Zapata and Crespo (1990); Zapata (2005); Zapata et al. (2007a, 2007b); Zapata et al. (2008); Fernández and Zapata (2011)
<i>Euglypha</i> sp. 1		No	No	Soil mosses	This taxon was reported by Hoogenraad and de Groot (1961) from moss samples (<i>Leucoloma billardieri</i> , [Brid.] Broth. S.) collected in Desolación Island, Angosta Port, Magellanic Strait, Tierra del Fuego Archipelago	CC	Hoogenraad and de Groot (1961)
<i>Euglypha</i> sp. 2		No	No	Soil mosses	This taxon was reported by Hoogenraad and de Groot (1961) from soil moss samples (<i>Campylopus introflexus</i> [Hedw.]) collected in south-central Chile	CC	Hoogenraad and de Groot (1961)
<i>Euglypha</i> sp. 3		No	No	Mosses and lichens covered by snow	This taxon was first described by Zapata et al. (2007b) from samples collected in south-central Chile	CC	Zapata et al. (2007b)
<i>Euglypha</i> sp. 4		No	No	Peatlands	This taxon was first described by Zapata et al. (2008) from samples collected in south-central Chile	CC	Zapata et al. (2008)
Scutiglypha	Fossner and Smet, 2001						
<i>Scutiglypha cabralae</i>	De Smet and Gibson, 2009	No	No	Arid shrublands, volcanic crater lakes	This species was first described by De Smet and Gibson (2009) from samples collected in the crater of Licancabur volcano. Later, this species was recorded near to the Atacama Desert, northern Chile (Fernández 2015). As the Licancabur volcano is situated in the political boundary between Chile and Bolivia, we think that this species could also occur in Bolivia. Therefore this species might be potentially endemic to the northern part of South America (at least northern Chile and south-central Bolivia)	NC, CC	De Smet and Gibson (2009); Fernández (2015)
<i>Scutiglypha crenulata</i>	Waller, 1912	No	No	Arid shrublands	This species was first reported by Fernández (2015)	NC, CC	Fernández (2015)
<i>Scutiglypha scutigera</i>	Penard, 1911	No	No	Arid shrublands	This species was first reported by Fernández (2015)	NC, CC	Fernández (2015)
..... Sphenoderidae	Chabouin et al., 2003						
Sphenoderia	Sakumuro et al., 1946						
<i>Sphenoderia fissirostris</i>	Penard, 1920	No	No	Acid mixed soils, conifer forest soils, humus on trunks, lakeshores, muddy soils, peatlands, pond sediments, rainforest mosses, rainforest soils, rainforest streams, soil mosses, soil humus	This species was first reported by Wallies (1913)	CC, SC	Wallies (1913); Jung (1942); Hoogenraad and de Groot (1961); Bonnet (1966)
<i>Sphenoderia macrolepis</i>	Leahy, 1979	No	No	Conifer forest soils, rainforest soils, soil humus	This species is first reported in the present study	CC, SC	Present study

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
	<i>Sphenoderia ovoides</i>	No	No	Rainforest mosses, rainforest streams, <i>Sphagnum</i> mosses, swamps	This species was first described by Jung (1942) from samples collected in southern Chile. This species is potentially endemic to southern Chile	CC, SC	Jung (1942)
	<i>Sphenoderia rimbophora</i>	No	No	Conifer forest soils, rainforest soils, soil humus	This species was first described by Bonnet (1966) from samples collected in southern Chile. This species also has been reported in Ecuador (Krashevskaya et al. 2007, although no pictures were provided). This species is potentially endemic to South America	CC, SC	Bonnet (1966)
	<i>Sphenoderia splendida</i>	No	No	Rainforest soils	This species was first reported by Jung (1942)	CC, SC	Jung (1942)
	<i>Sphenoderia valdiviana</i>	No	No	Rainforest soils, soil mosses	This species was first described by Chatelein et al. (2013) from samples collected in south-central Chile. This species is similar to <i>S. ovoides</i> Jung, 1942 and potentially could be a junior synonym of the latter. Further analyses are needed to confirm or reject this hypothesis (e.g. a morphometric and molecular comparison between these two species)	CC, SC	Chatelein et al. (2013)
	<i>Trachelocorythion</i>						
	<i>Trachelocorythion pulchellum</i>	<i>Corythium pulchellum</i> (Pérez) Tarasch, 1980	No	Rainforest soils, soil mosses	This species was first reported by Bonnet (1966) as <i>Corythium pulchellum</i> Penard, 1890	CC	Bonnet (1966)
.....	<i>Trinematidae</i>						
	<i>Corythion</i>						
	<i>Corythion conscripta</i>	<i>Trinema conscripta</i> (Cortés, 1989) Tarasch, 1980	No	Glacial rivers, pond sediments, rainforest soils, rainforest streams, soil mosses	This species was first described by Cortés (1989) as <i>Trinema conscripta</i> Cortés, 1989 from soil mosses collected in Cabo de Hornos southern Chile. Later, this species was reported by Jung (1942) and transferred to the genus <i>Corythion</i> Tarasch, 1981. According to Wallies (1913) this species could be a synonym of <i>C. dubium</i> Tarasch, 1981. However, Jung (1942) supported the taxonomic validity of this species. He emphasized that <i>C. conscripta</i> has a much thicker preapertural (upper) rim and larger size (more than twice) than <i>C. dubium</i> . Also, he argued that <i>C. conscripta</i> exhibits a wider ecological valence. Nonetheless, the last argument could be invalid since so far both species have been recorded in similar habitats in Chile. More recently, this species was also reported in southern Argentina by Vucelich and Lopretto (1995) which suggests that this taxon is valid. This species is potentially endemic to southern South America (southern Chile and southern Argentina)	CC, SC	Cortés (1989); Jung (1942)
	<i>Corythion delamarei</i>	No	No	Humus on trunks	This species was first reported by Bonnet (1966)	CC, SC	Bonnet (1966)
	<i>Corythion dubium</i>	No	<i>C. dubium aerophila</i> Decolre, 1950; <i>C. dubium unarmum</i> form Jung, 1942	Conifer forest soils, forest streams, humus in trunks, mosses and lichens covered by snow, mosses on trunks, pond sediments, rainforest mosses, rainforest soils, soil mosses	This species was first reported by Wallies (1913). Along with the typical form, Jung (1942) reported the occurrence of a undefined form. Bonnet (1966) reported the occurrence of <i>C. dubium aerophila</i> Decolre, 1950	CC, SC, AN	Wallies (1913); Jung (1942); Hocgenraad and de Groot (1951); Bonnet (1966); Smith (1978, 1984, 1985); Zapata et al. (2007b)
	<i>Corythion neboloides</i>	No	No	Rainforest soils	This species was first reported by Bonnet (1966)	CC	Bonnet (1966)
	<i>Corythion</i> sp.	No	No	Rainforest soils	This taxon was reported by Jung (1942) from samples collected in southern Chile	CC	Jung (1942)
	<i>Puytoracia</i>						
	<i>Puytoracia bergeri</i>	No	No	Volcanic crater glaciers	This species was first reported by Santibañez et al. (2010)	CC	Santibañez et al. (2011)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
	<i>Ptyotrachia jenswendtii</i>	Sanitbañez et al., 2011	No	Volcanic crater glaciers	This species was first described by Sanitbañez et al. (2010) from samples collected in a glacier situated inside of Osorno Volcano (south-central Chile). This species is potentially endemic to south-central Chile	CC	Sanitbañez et al. (2011)
Trinema	Dujardin, 1841						
<i>Trinema complanatum</i>	Perard, 1890	No	No	Acid mixed soils, and shrublands, conifer forest soils, humus in trunks, Maguelian forest soils, mosses and lichens covered by snow, mosses on trees, peatlands, pond sediments, rainforest mosses, rainforest soils, soil mosses, soil humus	This species was first reported by Jung (1942)	NC, CC, SC	Jung (1942); Fernández (2015)
<i>Trinema encheielys</i>	(Eichow, 1838)	<i>T. encheielys lineare</i> (Perard, 1890)	No	Arid shrublands, conifer forest soils, hot springs, humic soil lawns, humus in trunks, lake shores, Maguelian forest soils, Mallines, mosses and lichens covered by snow, muddy soils, on freshwater algae, on aquatic plants, peatlands, pond sediments, rainforest mosses, rainforest soils, sandy supralittoral, soil mosses, <i>Sphagnum</i> mosses, swamps, volcanic crater glaciers, volcanic crater lakes	This species was first reported by Certes (1889). Jung (1942) also reported the occurrence of <i>T. encheielys lineare</i> (Perard, 1890). Probably, Jung was referring to <i>T. lineare</i> Perard, 1890	NC, CC, CC, SC, EI, AN	Certes (1889); Wailes (1913); Jung (1942); Hoogenraad and de Groot (1951); Bonnet (1966); Smith (1978); Zapata and Matamala (1987); Zapata and Crespo (1990); Golemansky and Todorov (1996); Zapata et al. (2007b); Fernández and Zapata (2011); Sanitbañez et al. (2011); Fernández (2015)
<i>Trinema galeata</i>	(Perard, 1890)	No	No	Peatlands, pond sediments, rainforest soils, soil mosses, stream sediments	This species was first reported by Jung (1942)	CC, SC	Jung (1942); Bonnet (1966); Fernández and Zapata (2011)
<i>Trinema grandis</i>	(Charles, 1930)	No	No	Humic soil lawns, rainforest soils	This species was first reported by Bonnet (1966)	CC	Bonnet (1966)
<i>Trinema lineare</i>	Perard, 1890	No	No	Acid mixed soils, and shrublands, freshwater column, humic soil lawns, humus in trunks, lakeshores, Maguelian forest soils, peatlands, pond bottoms, rainforest soils, soil mosses, volcanic crater glaciers, volcanic crater lakes	This species was first reported by Wailes (1913)	NC, CC, CC, SC, EI, AN	Wailes (1913); Jung (1942); Hoogenraad and de Groot (1951); Smith (1978); Zapata and Matamala (1987); Zapata and Crespo (1990); Sanitbañez et al. (2011); Fernández (2015)
<i>Trinema savinellii</i>	Certes (1889)	No	No	Soil mosses	This species was first described by Certes from soil mosses collected in Cabo de Hornos southern Chile. Wailes (1913) suspected that this species was described based on a shell of a rotifer. As usual the description of this species is so poor that it is impossible to make a robust conclusion about its taxonomic validity. Wailes suggests considering this species as nomen nudum and we have followed his recommendation. This species has not been reported since its first description	SC	Certes (1889)
..... Incertae sedis Euglyphida: Euglyphidion	Adl et al., 2012						
Euglyphidion	Bonnet, 1960						
<i>Euglyphidion enigmaticum</i>	Bonnet, 1960	No	No	Humic soil lawns, rainforest soils	This species was first reported by Bonnet (1966). This genus is monospecific	CC	Bonnet (1966)
..... Incertae sedis Euglyphida: Pareuglyphia							
Pareuglyphia	Perard, 1902						
<i>Pareuglyphia reticulata</i>	Perard, 1902	No	No	Forest streams	This species was first reported by Jung (1942). Only two species worldwide (Meisterfeld 2002b)	CC, SC	Jung (1942)
..... Incertae sedis Euglyphida: Tracheuglyphia	Adl et al., 2012						
Tracheuglyphia	Dellandré, 1920						
<i>Tracheuglyphia acolia</i>	(Bonet and Thoma, 1955)	No	No	Acid mixed soils, humic soil lawns, mosses on trunks, peatlands, rainforest soils	This species was first reported by Bonnet (1966)	NC, CC, SC	Bonnet (1966)

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
...	<i>Tracheleuglypha dentata</i> (Petersen, 1880)	No	No	Arid shrublands, forest streams, mosses and lichens covered by snow, on aquatic plants, pond sediments, rainforest mosses, rainforest soils, lakeshores, stream sediments, <i>Sphagnum</i> mosses, swamps	This species was first reported by Jung (1942)	CC, SC	Jung (1942); Zapata et al. (2007b); Fernández (2015)
...	Incertae sedis Cercozoa: Psammonobiotidae						
...	Psammonobiotidae <i>Centropxyxiella</i> <i>Centropxyxiella arenaria</i>	<i>Golemansky, 1974</i> Valkarov, 1970 Valkarov, 1970	No No	Sandy supralittoral	This species was first reported by Golemansky and Todorov (1996). Some manuscripts (e.g. Meisterfeld 2002a) include this genus in Arcellinida (testate amoebae with lobopodia). Nonetheless, a recent revision of the genus revealed that <i>Centropxyxiella</i> Valkarov, 1970 is related to testate amoebae with filopodia and thus, might be included in the family Psammonobiidae Golemansky, 1974 (Golemansky and Todorov 2007)	SC	Golemansky and Todorov (1996)
	Chardezia <i>Chardezia caudata</i>	<i>Golemansky, 1970</i> Golemansky, 1970	No	Sandy supralittoral	This species was first reported by Golemansky and Todorov (1996). Traditionally, this genus has been included in the family Psammonobiidae (e.g. Meisterfeld 2002a; Golemansky and Todorov 2006) but a recent study (Golemansky and Todorov 2007) suggested that this genus would be a member of the family Cyphoderidae de Saeleer, 1934. However this suggestion needs to be supported by additional investigations (i.e. molecular data). This genus is monospecific	CC	Golemansky and Todorov (1996)
	Micranphora <i>Micranphora pontica</i> <i>Micranphora tokyoensis</i>	Valkarov, 1970 Valkarov, 1970 Sudzum, 1978	No No No	Sandy supralittoral Sandy supralittoral	This species was first reported by Golemansky and Todorov (1996) This species was first reported by Golemansky and Todorov (1996)	NC, CC, SC CC	Golemansky and Todorov (1996) Golemansky and Todorov (1996)
	Micropsammella <i>Micropsammella retorta</i>	Golemansky, 1970 Golemansky, 1970	No	Sandy supralittoral	This species was first reported by Golemansky and Todorov (1996)	CC	Golemansky and Todorov (1996)
	Ogdeniella <i>Ogdeniella elegans</i> <i>Ogdeniella taschevi</i>	Golemansky, 1982 Golemansky, 1970 (Golemansky, 1970)	No No No	Sandy supralittoral Sandy supralittoral	This species was first reported by Golemansky and Todorov (1996) This species was first reported by Golemansky and Todorov (1996)	CC, SC SC	Golemansky and Todorov (1996) Golemansky and Todorov (1996)
	Psammonobiotus <i>Psammonobiotus communis</i> <i>Psammonobiotus linearis</i> <i>Psammonobiotus minutus</i>	Golemansky, 1987 Golemansky, 1967 Golemansky, 1970 Golemansky, 1983	No No No No	Sandy supralittoral Sandy supralittoral Sandy supralittoral	This species was first reported by Golemansky and Todorov (1996) This species was first reported by Golemansky and Todorov (1996) This species was first reported by Golemansky and Todorov (1996)	SC SC CC	Golemansky and Todorov (1996) Golemansky and Todorov (1996) Golemansky and Todorov (1996)
...	Incertae sedis Cercozoa: Lenaella <i>Lenaella</i>	Auli et al., 2012 Hamm, 1988					

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
	<i>Lenaella neta</i>	<i>Hyalina neta</i> Jung, 1942	No	Sphagnum mosses	Jung (1942) first described this species as <i>Hyalina neta</i> Jung, 1942 from mosses collected in Calbuco Island, southern Chile. However, the genus <i>Hyalina</i> is a homonym of the genus <i>Lenaella</i> Haman, 1988 (see Haman, 1988). According to this reference, we synonymized this species as <i>Lenaella neta</i> (Jung, 1942). The genus <i>Hyalina</i> has been reported a few times in Europe but this taxon has never been used again since 1988 (Meisterfeld 2011, personal communication). Jung placed this species in the family Euglyphidae. However, according to the original description given by Jung (1942) this taxon lacks the typical characters found in other euglyphids (e.g. see Lara et al. 2007). We have placed this species out of Euglyphidae and provisionally in Cercozoa (incertae sedis) at least until its relationship with other members of Euglyphida is clarified. This species has not been observed again in Chile since its first description. This species could be potentially endemic to south-central Chile	OC, SC	Jung (1942)
...	Thecofilosea	Cavalier-Smith, 2000					
....	Incertae sedis Thecofilosea: Chlamydomphryidae	Adl et al., 2012					
....	Chlamydomphryidae	de Saedeleir, 1934					
....	Incertae sedis Chlamydomphryidae: <i>Clypeolina</i> , <i>Lecythium</i>						
	<i>Clypeolina</i>	Pennard, 1902					
	<i>Clypeolina marginata</i>	Pennard, 1902	No	Muddy soils, swamps	This genus and species are first reported in the present study	OC, SC	Present study
	<i>Lecythium</i>	Hentzel and C. Lissner, 1874					
	<i>Lecythium mutabilis</i>	[Bailey, 1855]	No	Pond sediments, stream sediments	This species was first reported by Wailes (1913) as <i>Pamphagus mutabilis</i> . However, the genus <i>Pamphagus</i> is a junior homonym of the genus <i>Lecythium</i> (Meisterfeld 2002b)	SC	Wailes (1913)
...	Incertae sedis Thecofilosea: Pseudodiffugiidae	Adl et al., 2012					
....	Pseudodiffugiidae	de Saedeleir, 1934					
	<i>Pseudodiffugia</i>	Schulmberger, 1945					
	<i>Pseudodiffugia fulva</i>	Antler, 1970	No				
	<i>Pseudodiffugia gracilis</i>	Schulthammer, 1946	No	Lakeshore mosses, Sphagnum mosses	This species is first reported in the present study	OC, SC	Present study
			<i>P. gracilis tenuicola</i> Bonnet and Thomas, 1960	Acid mixed soils, on freshwater algae, on trunks, peatlands, rainforest soils, rainforest streams, soil humus, soil mosses, Sphagnum mosses, swamps	This species was first reported by Wailes (1913). Later, Bonnet (1966) also reported the occurrence of <i>P. gracilis tenuicola</i> Bonnet and Thomas, 1960	OC, SC	Wailes (1913); Jung (1942); Bonnet (1966)
	<i>Pseudodiffugia</i> sp. 1		No	Soil mosses	This taxon was reported by Jung (1942) from soil moss samples collected in south-central Chile	OC	Jung (1942)
	<i>Pseudodiffugia</i> sp. 2		No	Pond sediments	This taxon was reported by Jung (1942) from pond sediment samples collected in south-central Chile	OC	Jung (1942)
	<i>Pseudodiffugia</i> sp. 3		No	Soil mosses	This taxon was reported by Jung (1942) from soil moss samples collected in south-central Chile	OC	Jung (1942)
	<i>Pseudodiffugia</i> sp. 4		No	Soil mosses	This taxon was reported by Smith (1978) from soil moss samples collected in the Antarctic	AN	Smith (1978)
•	Stramenopiles	Pennard, 1960					

	Taxonomic authority	Synonym	Subspecific taxa	Habitats	Additional notes	Distribution	References
..	Labyrinthulomycetes	Diak, 2001					
...	Incertae sedis Labyrinthulomycetes: Amphitremitidae	Adl et al., 2012					
....	Amphitremitidae	Poche, 1913					
	Archerella	Leobrich and Tappan, 1961					
	Archerella livum	Archerella livum Archerella livum	No	Estuaries, saltmarshes	This species was first reported by Zapata et al. (2002) as <i>Amphitrema livum</i>	CC	Zapata et al. (2002)
	Amphitrema	Archer, 1892	No	Peatlands	This species is first reported in the present study	SC	Present study
	Amphitrema stenostoma	Musell, 1884	No	Peatlands	This species is first reported in the present study	CC, SC	Present study
	Amphitrema wingthianum	Archer, 1899	No				
	Feuerbornia	Jung, 1942	No	Forest streams, rainforest soils	This genus is monospecific and was first described by Jung (1942) from samples collected in southern Chile. It seems that this species has also been reported in New Zealand (Meisterfeld 2002b). It is probably that this taxon exhibits a gondwanic distribution. This species was originally situated in the family Cyphoderiidae de Saedeleer, 1934. Then, Meisterfeld (2002b) transferred this genus to the family Amphitremitidae Poche, 1913 (incertae sedis). Later and without any apparent justification Adl et al. (2012) included this genus in the family Psammobiotidae Golemansky, 1974. However, a recent review of the family Psammobiotidae (see Golemansky and Todorov, 2007) does not even mention the genus <i>Feuerbornia</i> . Hence, we followed the suggestion of Meisterfeld (2002b) and situated this genus in the family Amphitremitidae. On the other hand, it should be noted that the inclusion of this genus in this family is still highly speculative and further investigations are needed	CC	Jung (1942)
	Feuerbornia lobophora	Jung, 1942	No				

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Chapter 3: Water-energy balance, past ecological perturbations and evolutionary constraints shape the latitudinal diversity gradient of soil testate amoebae in south-western South America



RESEARCH
PAPER

Water–energy balance, past ecological perturbations and evolutionary constraints shape the latitudinal diversity gradient of soil testate amoebae in south-western South America

Leonardo D. Fernández^{1,2*}, Bertrand Fournier³, Reinaldo Rivera², Enrique Lara¹, Edward A. D. Mitchell^{1,4} and Cristián E. Hernández²

¹Laboratory of Soil Biodiversity, Institute of Biology, University of Neuchâtel, Neuchâtel, 2000, Switzerland, ²Laboratorio de Ecología Evolutiva y Filoinformática, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario s/n, Concepción, Chile, ³Laboratoire Chrono-Environnement, UMR 6249 CNRS, Université Bourgogne Franche-Comté, Besançon Cedex 25030, France, ⁴Jardin Botanique de Neuchâtel, Neuchâtel 2000, Switzerland

*Correspondence: Leonardo D. Fernández, Laboratory of Soil Biodiversity, Institute of Biology, University of Neuchâtel, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland. E-mail: limnoleo@gmail.com

ABSTRACT

Aim To assess the largely neglected patterns and causes of latitudinal diversity gradients in soil protists, using South American testate amoebae as model organisms.

Location South-western South America (SSA).

Methods We assessed the relationship among richness, latitude and predictions of eight ecological hypotheses in SSA, a region characterized by harsh abiotic conditions northward and southward from mid-latitudes. We also examined the patterns of latitudinal variation in species range sizes, species composition and phylogenetic relatedness among species to assess whether the responses of species to current climate rely on evolutionary constraints.

Results Richness followed a unimodal trend with abrupt declines northward and southward from mid-latitudes. This trend was mostly explained by water–energy balance. To our knowledge, this mechanism has never been reported for soil microorganisms before. Species range sizes were larger northward and southward from mid-latitudes, a pattern which we have called the ‘two-way Rapoport effect’. Species composition was due to turnover at mid-latitudes and to nestedness towards low and high latitudes. Species at mid-latitudes tended to be less related to each other than those at low and high latitudes, supporting the existence of evolutionary constraints to water–energy availability.

Main conclusions The unimodal response of species diversity to water and energy availability stems from a combination of the ancestral adaptation of testate amoebae to warm, humid climates and past climate changes that set up permanent harsh abiotic conditions northward and southward from mid-latitudes. Given that other soil microorganisms also depend on water and energy to survive and reproduce, we predict that the unimodal response to water–energy balance observed will also be valid for a large number of microbial groups in SSA. While this diversity trend may not hold true for testate amoebae from other regions, we predict that their evolutionary constraints to water–energy availability will always produce strong relationships among richness, water and energy at large spatial-scales.

Keywords

Ecological hypotheses, hump-shaped diversity gradient, nestedness, Rapoport effect, soil microorganisms, species turnover, water–energy balance.

INTRODUCTION

The latitudinal diversity gradient is one of the most conspicuous patterns found for life on Earth. While classically the latitudinal diversity gradient predicts a decrease in species diversity from the tropics to the poles (Hillebrand & Azovsky, 2001), exceptions to this pattern are not uncommon in south-western South America (SSA), a narrow, long region situated in the Pacific margin of South America. Along this region several plants and animals exhibit a unimodal diversity gradient, characterized by a peak of richness at mid-latitudes and an abrupt pauperization at both low and high latitudes (see Appendix S1 in Supporting Information).

The origin of this unimodal diversity gradient is linked to the historical legacy of SSA, which has a rich history of geological and climate changes that are strongly associated with the distribution and diversification of its biota. During the Miocene, SSA was under a mesic climate with little seasonality and many Neotropical taxa were continuously distributed in this region (Villagrán & Hinojosa, 1997, 2005). However, during the Early Pleistocene, the Andes lifted rapidly, reaching elevations that exceeded 4000 m a.s.l., causing a strong rain shadow effect that led to the development of the Atacama Desert between c. 17–28° S (Gregory-Wodzicki, 2000). Moreover, from the Middle Pliocene onwards, a series of glacial advances and retreats characterized the landscape of austral SSA (Villagrán & Hinojosa, 1997, 2005). In particular, a single and continuous ice sheet covered broad areas ranging from c. 36–56° S during the Early Pleistocene, although coastal mid-latitude areas (c. 36–42° S) remained ice-free (McCulloch *et al.*, 2000). Notably, the apogee of this ice sheet coincided with an increase in the hyperarid conditions of the Atacama Desert (Villagrán & Hinojosa, 2005).

Under this scenario of drastic climatic changes, taxa at low and high latitudes became extinct, evolved into heat- or cold-tolerant species or were forced to migrate into ice-free mid-latitudes, which acted as a large refuge for them (Villagrán & Hinojosa, 1997, 2005). At mid-latitudes, glacial events also changed the drainage patterns, topography and even the continental divide, generating a highly diversified and fragmented mosaic of different biotopes that promoted allopatric speciation events (Moreno *et al.*, 2008; Arroyo *et al.*, 2004). Currently, mid-latitudes have high levels of biodiversity and endemism, constituting a representative part of the so-called 'Chilean biodiversity hotspot' (Arroyo *et al.*, 2004).

In summary, the dynamics of speciation and extinction, coupled with historical dispersal events and climatic oscillations, ultimately led to the formation of this unimodal diversity pattern characterized by a legacy of impoverished faunas and floras at low and high latitudes and a particularly plentiful diversity at temperate mid-latitudes. As the harsh environmental conditions still persist in low- and high-latitude areas, acting as an effective geographical barrier to biotic dispersal, and that there has been insufficient time period available for species to (re)colonize these areas, this regional hump-shaped diversity gradient persists to the present (Vil-

lagrán & Hinojosa, 2005; Moreno *et al.*, 2008; Samaniego & Marquet, 2009).

Traditionally, the assessment of this unimodal diversity gradient and its underlying causes has been focused exclusively on plants and animals. This raises the question of whether other fundamentally different life-forms, such as free-living unicellular eukaryotes (protists), also exhibit a unimodal diversity gradient across this region caused by the same aforementioned processes. Herein, we evaluated the latitudinal diversity gradient of soil testate amoebae (hereafter testate amoebae) and the causes of its origin and maintenance across SSA. Testate amoebae comprise a species-rich group of shelled eukaryotic microorganisms belonging to the supergroups Amoebozoa and SAR. Testate amoebae can be chiefly considered as a wet- and warm-adapted group for which water and temperature would be important constraints. Modern testate amoebae probably originated during the Upper Triassic (Schönborn *et al.*, 1999) under the warm, humid climates then prevailing at high palaeolatitudes (Preto *et al.*, 2010). They show higher growth, survival and reproductive success under conditions of high humidity and warm temperature (Laminger, 1978; Laybourn & Whyman, 1980). Although nowadays they can be found in almost all major terrestrial biomes on the planet, including extremely cold regions such as the Siberian tundra (Mazei & Chernyshov, 2011) or extremely hot ones such as the Atacama Desert (Fernández, 2015), testate amoebae are always more diverse in biomes characterized by high yearly rainfall and warm temperatures, (e.g. temperate rain forests; Bamforth, 2010; Fernández *et al.*, 2012). These facts, coupled with their well-known sensitivity to extreme temperatures (Schönborn, 1962) and water deficit (Harder *et al.*, 2016), led us to hypothesize that the need for high humidity and warm temperatures is a phylogenetically conserved trait in testate amoebae, and that the strength of this conservatism underlies their geographical distribution at large spatial scales.

Accordingly, we would expect that the set of historical events that established permanent harsh environmental conditions at both low and high latitudes in SSA should have played an important role in shaping the current diversity and distribution of testate amoebae via speciation, extinction and range changes. In particular, very few species should have developed wide climatic tolerances and crossed ecophysiological barriers to new northern and southern suboptimal environments. The diversity of testate amoebae, therefore, will exhibit a peak at temperate mid-latitudes and abrupt declines towards lower and higher latitudes (Prediction 1). Given the well-known physiological constraints in testate amoebae, it is also predicted that this diversity trend will be better explained by the joint availability of water and ambient energy in the environment (Prediction 2). Further, if the need for high humidity and warm temperatures is a phylogenetically conserved trait in these microorganisms, we would expect to find a set of congruent patterns of latitudinal variation in species range sizes, species composition and phylogenetic relatedness among species. In particular, these patterns should reflect the historical

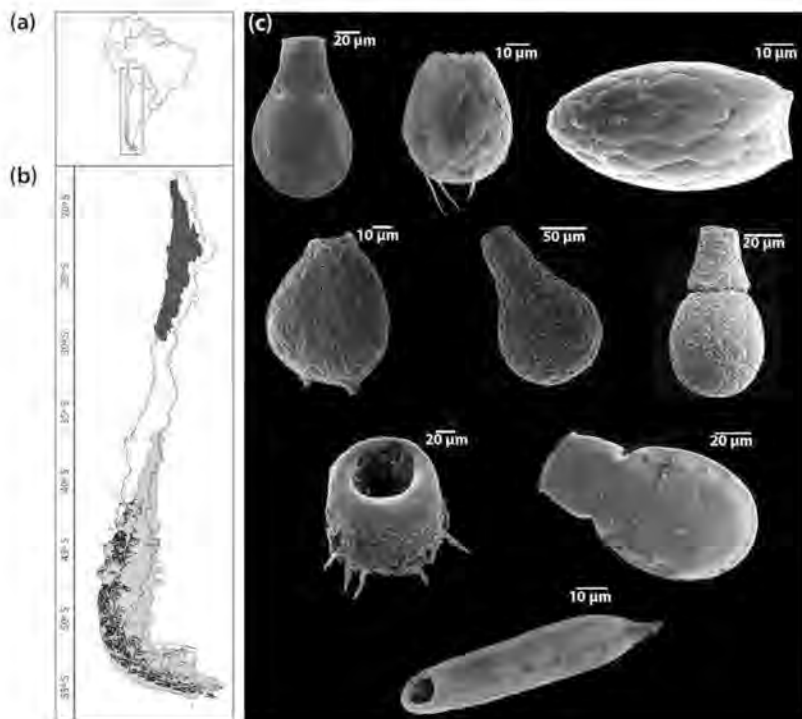


Figure 1 (a) Location and (b) map of the study region (a latitudinal gradient of c. 4350 km): the northern dark grey area shows the extent of the Atacama Desert (Gajardo, 1994) while the southern grey shaded area shows the extent of the ice sheet during the Last Glacial Maximum (McCulloch *et al.*, 2000). (c) Scanning electron microscope pictures of some testate amoeba species occurring in the study region. Top row (left to right): *Certesella martiali* (Certes, 1889); *Scutiglypha cabrolae* De Smet & Gibson, 2009; *Sphenoderia rhombophora* Bonnet, 1966. Second row (left to right): *Argyria schwabei* Jung, 1942; *Argyria gertrudeana* Jung, 1942; *Apodera vas* Certes, 1889. Third row (left to right): *Centropyxis aculeata* (Ehrenberg, 1838); *Alocodera cockayni* (Penard, 1910). Bottom: *Puytoracia jenswendti* Santibañez *et al.*, 2011. All pictures are from Fernández *et al.* (2015).

perturbations that settled permanent harsh environmental conditions at both ends of SSA. Accordingly, we predict that species range size distributions will: (1) fit a hollow curve (showing that most species have narrow latitudinal ranges in SSA); (2) show an increasing pattern from optimal to suboptimal environmental conditions (i.e. a Rapoport effect; Stevens, 1989), with larger latitudinal ranges northward and southward from mid-latitudes; and (3) exhibit a midpoint at mid-latitudes rather than at suboptimal lower and higher latitudes (Prediction 3). Furthermore, since areas with historically more stable climates promote higher diversification rates and biotic interactions (Mittelbach *et al.*, 2007), and the species composition of areas affected by past ecological perturbations is ultimately the result of recent (re)colonization of species coming from these latter areas (Moreno *et al.*, 2008; Hortal *et al.*, 2011; Rivadeneira *et al.*, 2011), variation in species composition will be mainly due to species replacement (i.e. turnover) at mid-latitudes and non-random exclusion of species (i.e. nestedness) within low and high latitudes (Prediction 4). Accordingly, species in temperate mid-latitudes will tend to be less related to each other than those situated northward and southward (Prediction 5). We used a comprehensive database on testate amoebae that summarizes distributional data gathered in SSA over more than 170 years to evaluate these five predictions.

METHODS

Data source

The testate amoeba dataset used in this study was obtained from Fernández *et al.* (2015). This study provides a compre-

hensive checklist as well as updated taxonomic, ecological and distributional information for all taxa of testate amoebae that have been recorded in SSA, Chile, from 1843 to the present (Fig. 1). The information included in the checklist was gathered from an exhaustive literature search, reviews of private and academic collections and several field expeditions conducted by the first author over the entire region since 2007. The information provided in the checklist summarizes over 170 years of data collection in SSA and currently represents the most complete and updated dataset for testate amoebae in this region. This study considers 215 of 252 species known for SSA. The remaining 37 species were excluded because they occur exclusively in aquatic biomes and/or have an uncertain taxonomic validity.

Latitudinal diversity gradient

Observed richness was calculated as the sum of all species recorded in each 1° latitudinal band. A coverage-based richness estimator (Chao *et al.*, 2014) was computed for each 1° latitudinal band to account for latitudinal bias in sampling effort. This method computes integrated curves that link rarefaction and extrapolation to estimate richness based on sampling completeness, which allows a less biased comparison of richness datasets. By contrast, other approaches estimate richness based on samples of equal size, which systematically biases the degree of differences between community richness (Chao *et al.*, 2014). Richness estimates and their 95% confidence intervals (95% CI) were computed using the package iNEXT (Hsieh *et al.*, 2014) implemented in R 3.1.2 (R Development Core Team, 2011). Appendix S2 gives details on the data used to conduct this approach.

Table 1 Ecological hypotheses tested with respect to the diversity of testate amoebae in south-western South America and their respective predictions and explanatory models.

Hypothesis	Prediction	Model parameters	Reference examples
1 Species–energy	Diversity positively related to energy inputs	Ultraviolet radiation B (UV-B) Potential evapotranspiration (PET)	Currie (1991), Evans <i>et al.</i> (2005)
2 Water availability	Diversity positively related to water inputs	Water deficit (WD) Annual precipitation (AP) Precipitation of the wettest month (PWM)	Rodriguez <i>et al.</i> (2005), Samaniego & Marquet (2009)
3 Water–energy balance	Diversity positively related to both water and energy inputs	Actual evapotranspiration (AET)	Hawkins <i>et al.</i> (2003), Rodríguez <i>et al.</i> (2005)
4 Productivity	Diversity positively related to energy inputs coming from plant production	Normalized difference vegetation index (NDVI)	Hutchinson (1959)
5 Habitat heterogeneity	Diversity positively related to environmental heterogeneity	Elevation (EL)	Pianka (1966), Kerr & Packer (1997)
6 Climatic variability	Diversity is positively related to more stable climates	Temperature seasonality (TS) Isothermality (IT)	Currie (1991), Kerr & Packer (1997)
7 Species–area	Diversity is positively related to land area	Average area in each 1° latitudinal band (AR)	Terborgh (1973), Rosenzweig (1995)
8 Mid-domain effect	Unimodal diversity within a bounded region (domain); highest diversity at mid-point of domain	Median number of species expected by a mid-domain null model (MDE)	McCain (2009) and references therein

Relationships among the observed and estimated richness with latitude were assessed by fitting different nonlinear models. The best-fitting model was selected based on the second-order Akaike information criterion (AIC_c) for each model. The AIC_c is a measure of relative model fit, proportional to the likelihood of the model and the number of parameters used to generate it. The best-fitting model is the one with the lowest AIC_c score. AIC_c scores are usually shown as ΔAIC_c , where the ΔAIC_c of a given model corresponds to the difference between its own AIC_c score and the AIC_c score of the best-fitting model (so the best model has a ΔAIC_c of zero). A model is considered implausible if its ΔAIC_c score is above 2, because this means that it loses too much information about full reality relative to other models under test (Burnham & Anderson, 2002). Nonlinear models and their individual 95% CIs were computed with *PAST* 3.08 (Hammer *et al.*, 2001). Prediction 1 is supported if a unimodal diversity gradient is recorded for both the observed and estimated richness.

Drivers of the current diversity

Relationships among the observed richness and the predictions of eight ecological hypotheses traditionally thought to drive large-scale diversity patterns (Table 1) were examined using ordinary least squares (OLS) regressions and simultaneous autoregression (SAR) to account for the potential spatial autocorrelation that may exist in the residuals (Kissling & Carl, 2008). Each hypothesis was represented by either a univariate or a multivariate explanatory model (Table 1). These models were constructed using a stepwise selection

procedure (Venables & Ripley, 2002), while the variables composing these models were calculated using different approaches (see Appendix S2 for further details). The fit and relative likelihood of each model were estimated using the ΔAIC_c score and Akaike weight (AIC_w). The outcomes of these analyses were contrasted with those of a principal component analysis (PCA) to look for independent lines of evidence for Prediction 2 (Appendix S2). OLS, SAR_{err}, ΔAIC_c and AIC_w analyses were computed using the R package *spdep* (Bivand *et al.*, 2013). Prediction 2 was supported if water and energy were selected as the major predictors of the species richness.

Historical legacy of testate amoeba diversity distribution

We assessed whether species range size distributions fit a hollow curve by analysing the relationship between the midpoint of the latitudinal range extent (MLRE) of all species co-occurring in the same 1° latitudinal band and richness by fitting different nonlinear models and using AIC_c to select the best model. Next, we assessed whether species range size distributions show an increasing pattern from optimal to sub-optimal environmental conditions (i.e. a Rapoport effect) by analysing the relationship between MLRE and latitude using piecewise regression to test the existence of a latitudinal species range size breakpoint at mid-latitudes. This analysis aims to find the point at which species move from having larger ranges to having shorter ranges. The breakpoint was found numerically by adopting a series of tentative breakpoints and performing a linear regression on both sides. The true

breakpoint was selected as the one having the largest coefficient of determination. Finally, we assessed whether species range size distributions exhibit a midpoint at mid-latitudes rather than at lower and higher latitudes. For this, we tested the geometrical shape (i.e. left triangle, right triangle, pyramid or inverted pyramid) that better predicted the data distribution for the relationship between the MLRE and the latitudinal range extent (LRE) of each species (i.e. a 'data distribution test') – where the limits of the geometrical shape may represent potential evolutionary constraints on range size (i.e. species cannot disperse beyond these limits) (Gotelli & Entsminger, 2001). Next, we calculated the sum of squares (SST) of the data points that fell within the selected geometrical shape to test whether they were significantly clustered away from or near to its boundaries (i.e. a 'boundary test'; 50,000 randomized matrices, $P < 0.05$). Nonlinear models and piecewise regressions were computed in *PAST* and *R*, respectively. Data distribution and boundary tests were computed in *ECO-SIM* 7.0 (Gotelli & Entsminger, 2001). Prediction 3 will be supported if species range size distributions exhibit (1) a hollow curve, (2) a breakpoint at mid-latitudes, and (3) a mid-point at mid-latitudes.

Multiple-site dissimilarity measures of beta diversity (Baselga, 2012) were computed to identify the phenomenon (i.e. turnover or nestedness) causing variation in species composition within low, mid- and high latitudes. Turnover (β_{SIM}) was calculated as the Simpson dissimilarity index, a metric that efficiently discriminates turnover from nestedness; while the pure effect of nestedness (β_{NES}) was estimated as the difference between the gamma-to-alpha diversity ratio (i.e. the total β -diversity) and turnover. These metrics range from zero (perfect similarity) to one (perfect dissimilarity). Differences in the number of latitudinal bands among areas of SSA were controlled for by resampling 10 1° latitudinal bands from each area 1000 times and computing the average β_{SIM} and β_{NES} . Low-, mid- and high-latitude areas were defined based on the maximum geographical limit of influence exerted by past climate changes at the north and the south of SSA (i.e. desertification and glaciation) (see Villagrán & Hinojosa, 2005). As β_{NES} is a measure of the compositional variation due to nestedness-resultant dissimilarity rather than a true metric of nestedness (Baselga, 2012), we assessed whether its results were consistent with those provided by NODF (for nestedness metric based on overlap and decreasing fill), an explicit metric for nestedness (Almeida-Neto & Ulrich, 2011). Multiple-site dissimilarity measures of beta diversity and nestedness were computed using the *R* package *betapart* (Baselga & Orme, 2012) and *NODF* 2.0 (Almeida-Neto & Ulrich, 2011), respectively. Prediction 4 is supported if variation in species composition produced by turnover at mid-latitudes and nestedness is found at both low and high latitudes.

Like many microorganisms, testate amoebae lack a comprehensive species-level time-calibrated phylogeny; this represents a major impediment to the systematic application of phylogenetic methods in ecology and biogeography. One way

to overcome this limitation is to use taxonomy as proxy for phylogeny. While the use of this approach does not allow a rigorous test of evolutionary predictions, it still can shed light on the relative importance of long-term processes in shaping species diversity gradients (McCain, 2009; Rivadeneira *et al.*, 2011). We used average taxonomic distinctiveness (AvTD) (Warwick & Clarke, 1998) as a proxy to assess the latitudinal variation of phylogenetic structure (i.e. the latitudinal variation of relationships between species) of testate amoebae. AvTD is a robust metric for assessing spatial trends of phylogenetic structure in organisms for which there are no phylogenetic reconstructions (Rivadeneira *et al.*, 2011; Winter *et al.*, 2013). AvTD measures the degree of inter-relatedness between species assuming that phylogenetic relationships are depicted across the structure of a taxonomy-based classification tree. Hence, AvTD quantifies the phylogenetic structure as the mean of all species-to-species distances through this topology for all pairs of species within a site (i.e. 1° latitudinal bands). Whenever possible, we used taxonomic hierarchies supported by recent phylogenetic analyses to compute AvTD. Invariant AvTD values (not departing from the predictions of the null model) would be related with the presence of assemblages composed of closely related species (phylogenetic clustering), revealing areas where environmental filtering would have a more important role than historical and/or evolutionary processes in the assembly of testate amoeba assemblages. Conversely, high AvTD values (above the null model) would highlight zones with assemblages composed of distantly related species (phylogenetic overdispersion), suggesting areas where long-term biotic interactions and evolutionary processes become relevant in the assembly of testate amoeba assemblages. AvTD was computed using *PRIMER* 6 (Clarke & Gorley, 2006). Prediction 5 is supported if we record AvTD values that are higher than expected at mid-latitudes.

RESULTS

Testate amoebae exhibited a unimodal diversity gradient with a peak of richness at mid-latitudes coupled with abrupt declines in richness towards lower and higher latitudes (Fig. 2a). This diversity gradient remained after correcting for latitudinal bias in sampling effort, thus supporting Prediction 1 (Fig. 2b). This diversity gradient was also better explained by the joint availability of water and energy in the environment [as measured by the actual evapotranspiration (AET); Table 2], giving support to Prediction 2. This result was consistent with those obtained with a PCA (Appendix S2).

Species range size distributions exhibited a hollow curve, revealing that most species have short–medium range extents in SSA (Fig. 3a). Next, the piecewise regression analysis showed that species range size distributions exhibit a breakpoint at 38.5° S. Therefore, testate amoebae exhibit both a decreasing (an inverted Rapoport effect; slope = -1.18 ± 0.06 , $R^2 = 0.96$, $P < 0.001$) and an increasing (a classical Rapoport effect; slope = 0.45 ± 0.14 , $R^2 = 0.46$, $P < 0.001$)

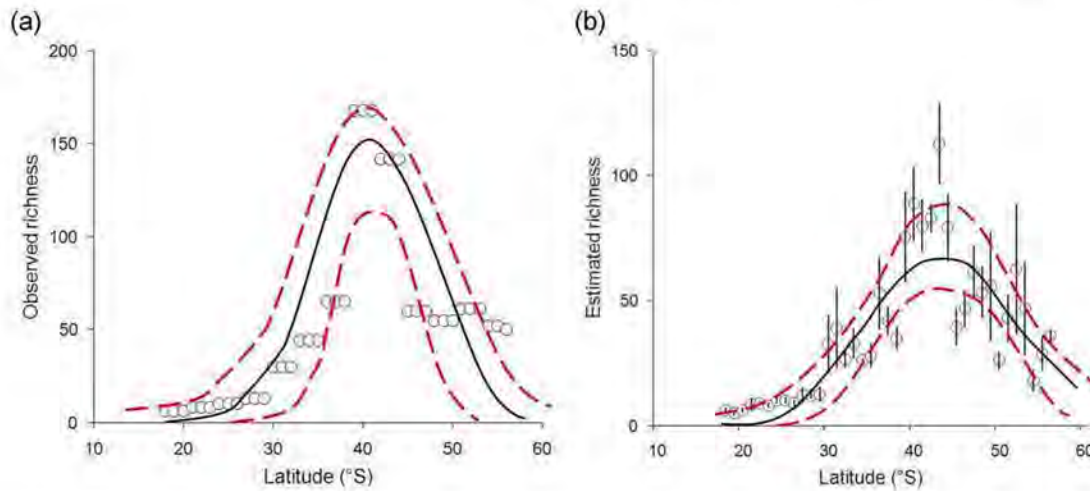


Figure 2 Latitudinal diversity gradient of soil testate amoebae in south-western South America. Unimodal relationship among latitude and (a) species richness observed and (b) species richness estimated with a coverage-based richness estimator (Chao *et al.*, 2014) for each 1° latitudinal. In both cases the fitted function (solid line) and its 95% confidence intervals (broken lines) are shown. In (b) the vertical solid lines show the 95% confidence intervals for the species richness estimated for each 1° latitudinal band.

species range size pattern below and above mid-latitudes, respectively (Fig. 3b). To our knowledge this is the first time that an inverted or a classical Rapoport effect (either separately or together) has been reported for soil microorganisms. This would also be the first time that the co-occurrence of both versions of the Rapoport effect (a pattern we have named the ‘two-way Rapoport effect’) has been reported. The data distribution resulting from the relationship between MLRE and LRE was better described by a pyramidal geometrical shape (Fig. 3c). The number of data points that fell within, and that were clustered at the centre of this geometrical figure, was higher than expected ($n = 199$ of 215 data points; $SST_{\text{observed}} = 124,620.70$, $SST_{\text{simulated}} = 117,369.60$; $P < 0.00001$), confirming that most species range size distri-

butions exhibit a mid-point at mid-latitudes. Altogether, these three outcomes supported Prediction 3.

Turnover was the reason for variation in species composition in mid-latitudes ($\beta_{\text{SIM}} = 0.61$, $P < 0.001$), though it played a less important role in low and high latitudes ($\beta_{\text{SIM}} = 0.14$ and 0.26 , respectively; $P < 0.001$) (Fig. 4a). By contrast, nestedness produced most of the variation in species composition in low- and high-latitudes ($\beta_{\text{NES}} = 0.52$ and 0.41 , respectively; $P < 0.001$) and displayed a minor role in mid-latitudes ($\beta_{\text{NES}} = 0.12$, $P < 0.001$) (Fig. 4b). These results agreed with those of NODF (Table 3), giving support to Prediction 4. Furthermore, species tended to be less related to each other at temperate mid-latitudes and closely related towards low and high latitudes (Fig. 5), which supports Prediction 5.

Table 2 Summary of the ordinary least squares (OLS) and simultaneous autoregressive model (SAR_{err}) regressions among testate amoeba species richness in south-western South America and the model of each ecological hypothesis under test.

			OLS regression		SAR _{err} regression				
Hypothesis		Model parameters	R ²	P-value	p-R ²	P-value	AIC _c	ΔAIC _c	AICw _i
1	Species-energy	UV-B, PET	0.44	< 0.001	0.88	< 0.001	359.65	8.39	0.01
2	Water availability	WD, AP, PWM	0.71	< 0.001	0.89	< 0.001	353.39	2.13	0.34
3	Water-energy balance	AET	0.69	< 0.001	0.88	< 0.001	351.26	0.00	0.59
4	Productivity	NDVI	0.53	< 0.001	0.89	< 0.001	356.55	5.29	0.04
5	Habitat heterogeneity	EL	0.36	< 0.001	0.89	< 0.001	360.22	8.95	0.01
6	Climatic variability	TS, IT	0.52	< 0.001	0.89	< 0.001	362.97	11.71	0.00
7	Species-area	AR	0.12	0.028	0.89	< 0.001	361.63	10.37	0.00
8	Mid-domain effect	MDE	0.22	0.003	0.89	< 0.001	359.89	8.62	0.01

The best fitting model was selected as the one having the lowest Akaike information criterion (as shown by a Δ AIC_c < 2) (Burnham & Anderson, 2002). Akaike weights (AICw_i) were also computed to assess that the model selected is actually the best explanatory model. See Table 1 for an explanation of the codes used for the explanatory variable in each model. R^2 values are not directly provided for SAR_{err} models, and thus model fit was assessed with a pseudo- R^2 value (p - R^2) calculated as the squared Pearson correlation between predicted and observed (i.e. true) values (Kissling & Carl, 2008).

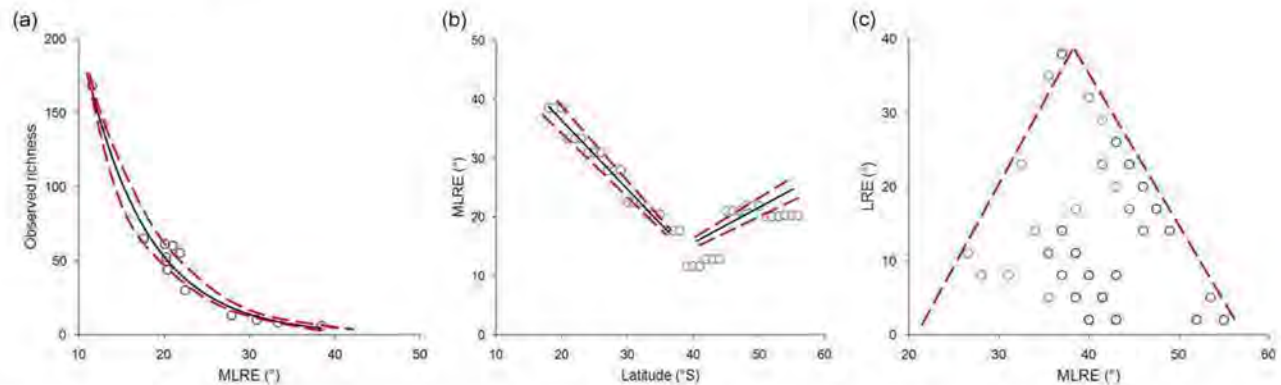


Figure 3 Species range size patterns recorded for testate amoeba species in south-western South America. (a) Relationship between the mean latitudinal range extent (MLRE) of all species co-occurring in the same 1° latitudinal band and richness. The fitted function (solid line) and the 95% confidence intervals (broken lines) of the nonlinear regression are shown. (b) Relationship between latitude and MLRE. The fitted function (solid line) and the 95% confidence intervals (broken lines) of the piecewise regression are shown. (c) Relationship between MLRE and the latitudinal range extent (LRE) of each species. Each point represents one species in the scatterplot ($n = 215$, but due to overlapping values some points are not visible). The scatterplot shows that the geometrical shape of the observed data distribution roughly fits a pyramid. The limits of the pyramidal shape (broken lines) represent potential evolutionary constraints that restrict species dispersal towards both lower and higher latitudes.

DISCUSSION

While for large organisms there is a growing recognition that broad-scale richness patterns are determined by a balance between ecological and long-term processes (Mittelbach *et al.*, 2007), there is a long-standing, and yet unresolved, debate on whether microbial diversity patterns also originated from, and are maintained by, these same processes at large spatial scales (Martiny *et al.*, 2006). Recent studies have shown that eukaryotic microbial diversity covaries with latitude and that ecological variables are drivers of this relationship (e.g. Hillebrand & Azovsky, 2001; Azovsky & Mazei, 2013; Bates *et al.*, 2013). However, hitherto, the possible influence of long-term processes in shaping the spatial patterns of protist diversity has been largely neglected (Martiny

et al., 2006; Fontaneto, 2011). Furthermore, most studies analysing broad-scale protist diversity patterns are biased towards aquatic taxa (i.e. ciliates, diatoms) and soil protists remain poorly documented (Foissner, 1999; Chao *et al.*, 2006; Smith *et al.*, 2008; Bobrov *et al.*, 2015). Our study sets a new trend by analysing not only the importance of ecological variables but also the possible role played by long-term processes in shaping the latitudinal diversity patterns of testate amoebae and soil protists in general. Herein, we propose that the need for high humidity and warm temperatures is a phylogenetically conserved trait in testate amoebae, a hypothesis that integrates both ecological and long-term processes to explain diversity patterns (Wiens *et al.*, 2010).

Like many plants and animals in SSA (Appendix S1), testate amoebae exhibit a unimodal diversity gradient

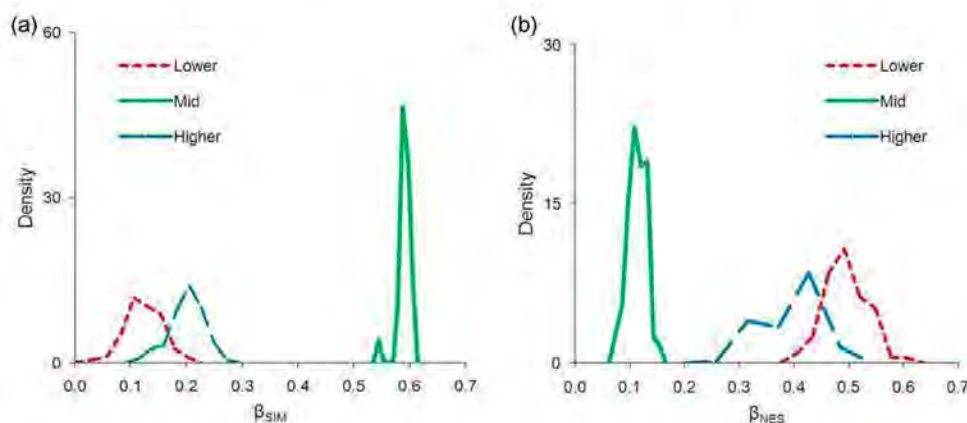


Figure 4 Processes underlying variation in testate amoeba species composition in low-, mid- and high-latitude areas of south-western South America. In mid-latitudes (a) turnover (β_{SIM}) is the most important process in generating variation in species composition whereas in lower and higher latitudes (b) it is nestedness (β_{NES}). Kernel density curves were constructed by resampling 10 1° latitudinal bands from each area 1000 times and computing the average β_{SIM} and β_{NES} .

Table 3 Degree to which testate amoeba assemblages at low, mid- and high latitudes in south-western South America exhibit a nested subset pattern of species composition.

	NODF _{OBS}	NODF _{EXP} ($\pm$ SD)	L95% CI	U95% CI	P-value
NODF _{LOW}	54.51	52.06 ($\pm$ 0.03)	51.58	53.01	0.001
NODF _{MID}	55.30	56.99 ($\pm$ 0.10)	56.75	57.09	0.001
NODF _{HIGH}	53.92	50.92 ($\pm$ 0.09)	50.75	51.08	0.001

Nestedness was based on a binary matrix ordered according to the marginal row/column sums. Below are shown the observed (NODF_{OBS}) and average expected [NODF_{EXP} ($\pm$ SD)] values of the NODF index for low- (NODF_{LOW}), mid- (NODF_{MID}) and high-latitude (NODF_{HIGH}) areas. NODF ranges from 0 to 100, where high values are consistent with higher degrees of nestedness. The statistical inference was based on the lower and upper confidence intervals (L95% CI and U95% CI) of the null distribution of 50,000 randomized matrices. NODF_{OBS} values higher than expected by chance indicate areas where spatial variation in species composition is chiefly due to a non-random and progressive loss of species towards one of the ends of the latitudinal gradient studied (i.e. NODF_{LOW} and NODF_{HIGH}). Instead, NODF_{OBS} values lower than expected by chance point out areas where spatial variation in species composition is mainly due to a non-random species turnover (i.e. NODF_{MID}).

characterized by a peak of richness at temperate mid-latitudes and steep declines in richness towards low and high latitudes (Fig. 2a). A correlation analysis conducted between richness estimates for each 1° latitudinal band and latitude revealed the same relationship (Fig. 2b). This relationship remained even after accounting for latitudinal bias in sampling effort (Fig. 2b), suggesting that this trend must be truly representing the latitudinal diversity pattern for testate amoebae in SSA and their limited physiological capacity to adapt

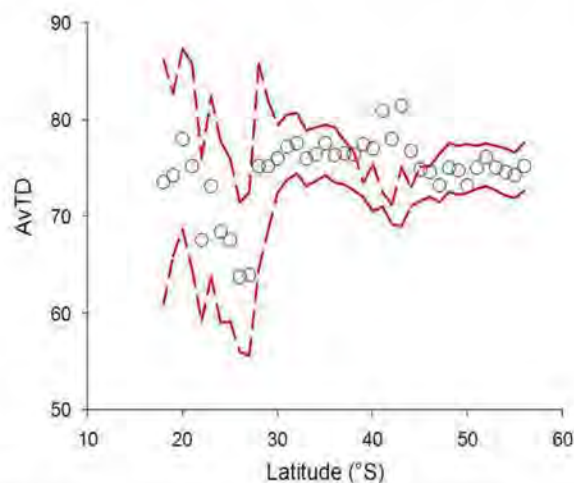


Figure 5 Latitudinal variation of testate amoeba average taxonomic distinctiveness (AvTD) values (circles) along south-western South America. The broken lines show the lower and upper 95% prediction curves of a null model built by resampling the AvTD values 10,000 times.

to the harsh environmental conditions that prevail northward and southward of mid-latitudes.

Water, in combination with energy availability (as measured by AET), was the major predictor of this unimodal diversity trend (Table 2). To our knowledge this is the first time that such a relationship, known as water–energy balance (*sensu* Hawkins *et al.*, 2003), has been explicitly tested and reported for testate amoebae and soil protists in general. While this finding does not rule out the role of other ecological factors as drivers of current testate amoeba diversity at large spatial scales, it certainly identified water and energy as the most important among those considered. A PCA (Appendix S2) confirmed water and energy variables as primary predictors of testate amoeba richness in SSA, though it also showed that the observed unimodal response to water and energy availability is not easy to separate from other so-called drivers of diversity such as (plant) productivity. Most likely this is because most surrogates for productivity (and other climatic-based causes) can be also seen as proxies for water and energy availability (Hawkins *et al.*, 2003; Rodríguez *et al.*, 2005). This calls for attention, especially when attempting to determine whether the reason behind the observed climate–richness links is physiologically based water–energy balance or trophically based productivity (Hawkins *et al.*, 2003). For testate amoebae, however, this may not be an issue. Water and energy are undoubtedly essential for physiological processes in testate amoebae (Schönborn, 1962) and have direct effects on their growth, survival and reproductive success (Laminger, 1978; Laybourn & Whyman, 1980), as well as on their abundance, diversity and distribution at large spatial scales (Wilkinson, 1994; Bates *et al.*, 2013; Lara *et al.*, 2016; present study). By contrast, productivity is probably much more important at local spatial scales, especially when water and energy (as measured by soil moisture and temperature) are not limiting variables (Fournier *et al.*, 2016; Harder *et al.*, 2016). Furthermore, it is expected that the importance of energy or water availability will vary latitudinally throughout SSA: the diversity of testate amoebae will be better explained by ambient energy at high latitudes because ambient energy levels are lower in these areas, while water will be more important at low latitudes because ambient energy is higher in these areas and thus not life-limiting. This prediction would agree with a study showing that temperature (as a surrogate for energy) is a strong predictor of nebelid testate amoeba diversity at higher latitudes in SSA (Wilkinson, 1994). However, it worth mentioning that the aforementioned study was based on limited data for testate amoebae and water availability, and accordingly, this prediction remains untested.

The existence of a balance between water and energy inputs can be traced back in time for temperate mid-latitudes. Fossil records indicate that this region has supported a flora adapted to warm, humid climates at least since the Palaeocene (Villagrán & Hinojosa, 1997, 2005). This favourable long-standing climatic stability may explain not only the peak of testate amoeba richness in this zone but

also their set of interrelated patterns of species range size in SSA (Fig. 3). Climatic stability reduces the chances of extinction, facilitates the accumulation of species over the time and promotes stronger biotic interactions that lead to greater specialization and shorter geographical ranges (reviewed in Mittelbach *et al.*, 2007). For testate amoebae, this effect may be exacerbated by their shorter generation times (Laybourn & Whymant, 1980) and low tolerances for extreme temperatures (Schönborn, 1962) and water deficit (Harder *et al.*, 2016), which probably enhanced their diversification and isolation in the comparatively more stable mid-latitudes during the development of the Atacama Desert in the north and glacial advances in the south (Villagrán & Hinojosa, 2005). Support for this stems from the unusually high concentration of short-ranged testate amoeba species in mid-latitudes (i.e. as shown by a hollow curve and the two-way Rapoport effect), which seems to be related to the high local endemism of these areas (Fernández *et al.*, 2015), and the fact that most species exhibit a MLRE at mid-latitudes (Fig. 3), reinforcing the idea that temperate areas of SSA exhibit a combination of environmental conditions (a water–energy balance) that are optimal for growth, survival and reproduction of these microorganisms. It is worth mentioning that this does not rule out the possible existence of endemic species of testate amoebae in areas with harsh abiotic conditions. In SSA, a few potentially endemic species occur only at low and high latitudes (Fernández *et al.*, 2015) and exhibit a MLRE outside temperate areas (present study, Fig. 3). However, the low endemism and diversity of these areas suggest that these few species represent cases of recent adaptation to harsh abiotic conditions.

Past climate changes that set up the current climate of SSA also left their signal on latitudinal variation in the species composition of testate amoeba. This variation was produced by turnover at mid-latitudes (species are replaced by others from site to site resulting in species-rich areas) and by nestedness towards low and high latitudes (species are gradually excluded, and not replaced by others, northward and southward from mid-latitudes, resulting in progressively species-poorer areas) (Fig. 4, Table 3). Over broad spatial scale turnover is associated with areas that promoted species persistence during past climate changes and that currently concentrate high levels of biodiversity and endemism; while nestedness is linked to recent and limited recolonization of areas where species were wiped out by historical perturbations (Moreno *et al.*, 2008; Hortal *et al.*, 2011; Rivadeneira *et al.*, 2011). So, the turnover observed at mid-latitudes suggests that testate amoeba species persisted in these areas during the past climate changes that affected the north and the south of SSA. Since these climatic events also reduced the number of proper habitats in mid-latitudes (Arroyo *et al.*, 2004), it is very likely that there they also underwent allopatric speciation events (Zapata and Fernández, 2008), which finally led to the high diversity and endemism rates currently found in these areas. Likewise, the high level of nestedness observed towards the north and the south of SSA, coupled

with the occurrence of northern and southern species-poor assemblages mainly composed of small generalist species (Fernández *et al.*, 2015), suggests that species composition in these areas is mainly the result of post-disturbance recolonization by species with a wide tolerance to harsh abiotic conditions coming from benign environments situated at mid-latitudes. Small generalist species are thought to be among the earliest colonizers of new habitats (Lara *et al.*, 2011; Fournier *et al.*, 2016) and of previously glaciated areas (Wilkinson, 1990; Carlson *et al.*, 2010; Fernández *et al.*, 2012), which also suggests that the recolonization of northern and southern areas is a recent and still ongoing process. Dispersal capability does not play a significant role in limiting the progressive recolonization of testate amoebae towards northern and southern areas. At least the smaller species are good dispersers and, theoretically, could overcome major physical barriers to dispersal in SSA, for example the Atacama Desert and glaciers to the north and south, respectively, in short periods of time (e.g. 1 year) (Wilkinson *et al.*, 2012). Rather, their phylogenetically conserved response to water and temperature must prevent most species from colonizing extra-temperate areas, and most likely still limits the chances of reproduction and survival of those that have already colonized such areas. Populations of species situated northward and southward are thus probably non-viable (i.e. individuals may survive but can hardly reproduce) and permanently 'rescued' from extinction by the immigration of individuals coming from viable populations located in mid-latitude areas with optimum abiotic conditions (i.e. a rescue effect; Brown & Kodric-Brown, 1977).

In further support of the previous idea, species in northern and southern assemblages are more closely related to each other than those in mid-latitudes (Fig. 5). Assemblages of closely related species result from the effects of environmental filtering, where harsh environmental conditions select a species subset from a regional pool based on their climatic tolerances; while assemblages of distantly related species are often the result of accumulation of specialized species via diversification in areas that have historically exhibited the ancestral climatic conditions within which those taxa evolved (Wiens *et al.*, 2010). This outcome reinforces our argument that the unimodal diversity pattern observed for testate amoebae stems from their evolutionary history coupled with the historical legacy of SSA. The interaction between their narrow physiological constraints and the longstanding climatic stability of mid-latitudes (Villagrán & Hinojosa, 2005) is likely to have promoted species coexistence via competitive exclusion and diversification of species specialized in the use of different resources in warm and humid environments. However, other mechanisms such as facilitative interactions among distantly related species cannot be discarded (Day & Young, 2004). Moreover, the current severe northern and southern abiotic conditions are probably 'filtering' only species that have a similar phylogenetically constrained thermal and humidity tolerance, resulting in assemblages of closely related species.

CONCLUDING REMARKS

In SSA, the unimodal response of species diversity to current water and energy availability stems from a combination between the ancestral adaptation of testate amoebae to warm, humid climates and past climate changes that affected areas northward and southward of mid-latitudes. Given that other soil microorganisms also depend on water and energy to survive, grow and reproduce, we predict that the observed unimodal response to water–energy balance will also be valid for a large number of microbes in SSA.

Because the historical legacy of SSA is unique in terms of geological and climate changes, we do not expect that the unimodal response of species diversity to water–energy balance observed here will be valid for all regions of the planet. However, we do expect that the phylogenetically conserved response to warm and humid climates proposed here for testate amoebae will also hold true in other regions. Accordingly, we predict that regardless of the trend followed by their diversity within a particular region (e.g. an increasing or decreasing trend), the diversity of testate amoebae will always be better predicted by water and energy at large spatial scales. This prediction naturally has a caveat: the influence of water will probably decrease and the influence of energy will increase with absolute latitude.

Finally, the unimodal response of species diversity to water–energy balance reaffirms the usefulness of testate amoebae as proxy indicators of climate change, while their conserved response to warm and humid climates suggests that the processes that originate and maintain diversity in large organisms also could operate in microbes.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Appendix S1 Plants and animals exhibiting a unimodal diversity gradient in the study site.

Appendix S2 Additional data and analyses used to support the predictions of this study.

BIOSKETCH

Leonardo D. Fernández is a joint PhD student at the Universidad de Concepción (Chile) and the University of Neuchâtel (Switzerland). He is using approaches in biogeography, macroecology and landscape genetics to clarify the processes that originate and maintain microbial diversity patterns at different spatial-scales.

Author contributions: L.D.F. and C.E.H. conceived the idea; L.D.F. and B.F. performed the statistical analyses; R.R. contributed data on environmental variables; L.D.F. wrote a draft of the paper, and all authors contributed substantially to revisions.

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Appendix to “Water-energy balance, past ecological perturbations and evolutionary constraints shape the latitudinal diversity gradient of soil testate amoebae in south-western South America”

SUPPORTING INFORMATION

Water–energy balance, past ecological perturbations and evolutionary constraints shape the latitudinal diversity gradient of soil testate amoebae in southwestern South America

Leonardo D. Fernández, Bertrand Fournier, Reinaldo Rivera, Enrique Lara,
Edward A.D. Mitchell and Cristián E. Hernández

Appendix S1 Plants and animals exhibiting a unimodal diversity gradient in the study site.

Table S1 Animals and plants with a unimodal diversity gradient in Southwestern South America.

Taxa	References
Vascular and non-vascular plants (trees, shrubs, vines, mosses, ferns, lycophytes)	Villagrán & Hinojosa (1997, 2005); Bannister <i>et al.</i> (2012); Segovia <i>et al.</i> (2013); Parra <i>et al.</i> (2015)
Marine nematodes	Lee & Riveros (2012)
Insects (Diptera, Ephemeroptera, Lepidoptera, Plecoptera, Trichoptera)	Löwenberg-Neto <i>et al.</i> (2008); Samaniego & Marquet (2009); Palma (2013)
Freshwater mollusks (gastropods and bivalves)	Valdovinos (2006a); Fuentealba <i>et al.</i> (2010)
Freshwater crustaceans	Valdovinos (2006b)
Polychaetes	Hernández <i>et al.</i> (2005); Moreno <i>et al.</i> (2006, 2008)
Fishes (intertidal and subtidal fishes)	Navarrete <i>et al.</i> (2014)
Birds	Vilina & Cofré (2006)
Mammals	Cofré & Vilina (2006); Samaniego & Marquet (2009)

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SUPPORTING INFORMATION

Water–energy balance, past ecological perturbations and evolutionary constraints shape the latitudinal diversity gradient of soil testate amoebae in southwestern South America

Leonardo D. Fernández, Bertrand Fournier, Reinaldo Rivera, Enrique Lara,
Edward A.D. Mitchell and Cristián E. Hernández

Appendix S2 Additional data and analyses used to support the predictions of this study.

Statistical procedure and data used to conduct a coverage-based rarefaction-extrapolation procedure

A coverage-based rarefaction-extrapolation procedure (Chao *et al.*, 2014) was conducted for each 1° latitudinal band to assess whether the latitudinal diversity gradient observed would appear after accounting for sampling bias. This method computes integrated curves based on sampling theory that smoothly link rarefaction and extrapolation. These curves estimate species richness based on a standardized measure of sampling completeness, allowing a less biased comparison of richness datasets. By contrast, traditional rarefaction and extrapolation approaches compare richness data of a set of communities of equal size, which systematically biases the degree of differences between community richnesses (Chao *et al.*, 2014). This method requires information on both the number of samples collected at each 1° latitudinal band and the incidence (presence or absence) of each species in each sampling unit (i.e. also known as sample-based incidence data). This information was obtained from Fernández *et al.* (2015), a study providing a comprehensive checklist as well as updated taxonomic, ecological and distributional information for all testate amoeba taxa that have been recorded in Southwestern South America, since 1843 to 2014. The information included in that study was gathered from different sources including literature, private and academic collections, and field expeditions conducted by the first author of the present study all over the study site between 2007 and 2014. Given

that the information required by the species estimator was rarely available from all the sources (i.e. number of samples per latitudinal band plus sample-based incidence data); we based our analysis only on the samples collected in field expeditions by the first author ($n = 6710$ samples). These samples were chosen because they were collected and treated by the same person under standardized methods and thus, we have a known number of samples per latitudinal band and species incidences per sample unit. These samples were taken at different altitudes, including sites located at sea level and in the Andes, as well as all major terrestrial biomes on the study site such as the Atacama Desert, grasslands and the temperate rain forest among others. A sample was defined as a unique portion of 10-cm³ of soil, moss or litter collected in 1° latitudinal band, while the total number of samples collected within each 1° latitudinal band was referred to as the '*reference sample*'. Reference samples were used to compute for each latitudinal band a species richness estimate up to the larger reference sample (Latitude 42° S, Reference sample = 858 samples) following Chao *et al.* (2014). The table summarising the information used to compute diversity estimates is shown below in the form of a sample-by-species incidence matrix for each 1° latitudinal band following Colwell *et al.* (2012), where '*i*' is the incidence of a given species in each sampling unit (Table S1). For example, in the latitude 18° S, an '*i*' value of 158 means that the species 1 was found in 158 of 215 samples. Detailed information on the species recorded at each 1° latitudinal band is given in Fernández *et al.* (2015).

Table S1 Sample-by-species incidence matrix for each 1° latitudinal band used to conduct a coverage-based rarefaction and extrapolation procedure.

Latitude 18° S, Reference sample = 215, Species observed = 6
<i>i</i> = 158-68-32-21-5-1
Latitude 19° S, Reference sample = 100, Species observed = 5
<i>i</i> = 91-51-28-22-1
Latitude 20° S, Reference sample = 105, Species observed = 6
<i>i</i> = 81-77-65-65-21-5
Latitude 21° S, Reference sample = 48, Species observed = 8
<i>i</i> = 39-31-25-15-12-8-2-1
Latitude 22° S, Reference sample = 42, Species observed = 8
<i>i</i> = 40-35-25-22-19-12-1-1
Latitude 23° S, Reference sample = 85, Species observed = 8
<i>i</i> = 81-75-66-52-33-13-12-2

Latitude 24° S, Reference sample = 123, Species observed = 10
$i = 101-75-63-58-46-33-25-5-3-1$
Latitude 25° S, Reference sample = 78, Species observed = 10
$i = 72-62-48-25-23-12-7-5-4-1$
Latitude 26° S, Reference sample = 250, Species observed = 9
$i = 210-101-88-56-35-18-12-4-1$
Latitude 27° S, Reference sample = 300, Species observed = 12
$i = 82-62-58-35-29-15-12-8-3-2-2-1$
Latitude 28° S, Reference sample = 66, Species observed = 12
$i = 41-38-33-25-21-18-18-6-6-5-4-1$
Latitude 29° S, Reference sample = 29, Species observed = 13
$i = 38-19-18-14-13-12-9-9-8-6-5-2-1$
Latitude 30° S, Reference sample = 120, Species observed = 25
$i = 98-84-65-63-58-45-35-34-27-25-13-12-12-10-8-7-6-6-5-3-2-1-1-1-1$
Latitude 31° S, Reference sample = 88, Species observed = 27
$i = 68-63-61-52-51-45-42-32-25-24-18-15-15-13-12-9-8-6-4-4-3-2-1-1-1-1-1$
Latitude 32° S, Reference sample = 210, Species observed = 26
$i = 98-81-66-52-51-45-43-42-37-33-32-31-28-21-19-19-18-17-15-12-6-5-5-3-2-1$
Latitude 33° S, Reference sample = 62, Species observed = 31
$i = 51-33-32-32-31-26-25-25-21-19-19-19-18-17-17-16-16-15-13-12-9-6-6-5-5-5-3-2-1-1$
Latitude 34° S, Reference sample = 91, Species observed = 26
$i = 81-77-41-36-25-25-23-22-21-21-21-20-19-16-15-14-14-14-13-12-12-12-11-11-10-1$
Latitude 35° S, Reference sample = 198, Species observed = 26
$i = 100-98-67-55-51-41-18-18-17-14-14-14-13-13-13-12-12-12-11-9-5-4-3-2-1-1$
Latitude 36° S, Reference sample = 62, Species observed = 45
$i = 52-51-33-32-32-32-28-26-25-24-23-21-20-19-18-17-16-16-15-15-15-15-14-12-12-11-10-9-9-9-8-8-7-6-5-5-5-4-3-2-1-1-1-1$
Latitude 37° S, Reference sample = 120, Species observed = 39
$i = 91-85-78-66-52-42-38-37-32-26-25-25-24-24-22-22-21-20-19-16-15-9-9-8-7-6-5-5-5-4-3-2-2-2-1-1-1-1-1$
Latitude 38° S, Reference sample = 65, Species observed = 32
$i = 55-41-41-39-36-35-26-25-25-17-16-16-15-15-15-13-12-12-11-10-10-9-9-8-8-7-6-5-5-1-1-1$
Latitude 39° S, Reference sample = 86, Species observed = 65
$i = 81-65-64-63-62-55-55-55-41-41-41-33-32-30-28-28-25-25-19-19-18-16-16-15-15-15-15-14-13-12-12-11-11-10-10-10-9-9-8-7-7-6-6-5-5-5-5-5-4-4-4-3-2-2-2-1-1-1-1-1-1-1$
Latitude 40° S, Reference sample = 110, Species observed = 81
$i = 98-88-77-76-71-66-65-64-62-56-55-55-52-51-50-48-42-38-36-32-31-25-23-18-17-17-17-16-16-16-16-15-15-15-15-15-14-13-13-13-12-12-12-12-11-11-11-11-10-10-10-10-9-9-9-9-8-8-7-7-6-6-6-6-6-6-5-5-5-5-5-4-4-4-3-2-1-1-1-1-1$
Latitude 41° S, Reference sample = 258, Species observed = 73
$i = 201-110-99-91-81-75-71-71-69-68-66-65-55-55-52-51-38-36-33-32-31-29-25-25-23-22-21-19-18-18-17-15-15-14-9-9-9-8-8-8-8-8-7-7-7-6-6-6-6-6-6-5-5-5-5-5-5-4-4-3-3-2-2-2-2-1-1-1-1-1-1-1$
Latitude 42° S, Reference sample = 858, Species observed = 83
$i = 326-225-189-145-87-84-81-74-71-69-69-66-66-65-63-62-60-59-59-58-58-55-51-48-46-41-38-34-32-28-27-27-26-26-26-26-26-26-25-25-25-25-25-25-25-25-24-24-24-24-23-23-22-21-21-20-14-10-9-9-8-8-8-7-7-7-7-6-5-5-4-1-1-1-1-1-1-1-1-1-1$

Statistical procedure followed to calculate the variables composing the models of the ecological hypotheses tested in this study

To assess the effects of present-day spatial and environmental factors in shaping the latitudinal gradient of soil testate amoebae in southwestern South America, we selected seventeen classical explanatory variables to construct both univariate and multivariate candidate models per each of the eight ecological hypotheses under test. All variables (excepting mid-domain values which were directly calculated with a specific program) were re-sampled from different sources (see below), using a resolution of $1^{\circ} \times 1^{\circ}$. This procedure generated $1^{\circ} \times 1^{\circ}$ cells within each 1° latitudinal band (i.e. latitudinal $1^{\circ} \times 1^{\circ}$ cells) with information for each of the seventeen variables. The information contained within the latitudinal $1^{\circ} \times 1^{\circ}$ cells was then averaged to get an overall value per each 1° latitudinal band. The re-sampling procedure and arithmetic mean calculations were performed using ArcGIS 10.2 (ESRI, 2014). Note that some of the variables described below were not used to construct the explanatory models showed in the main body of the manuscript because, according to an AIC-based stepwise selection procedure, they were non-significant in driving testate amoeba diversity patterns.

The hypotheses and their associated variables were:

- 1) Species–energy: We tested this hypothesis using ultraviolet radiation B (UV-B), potential evapotranspiration (PET), and annual mean temperature (AMT). UV-B is available at Beckmann *et al.* (2014), PET (1950-2000) at <http://www.csi.cgiar.org> (Trabucco & Zomer, 2009), and AMT at <http://www.worldclim.org/> (Hijmans *et al.*, 2005).
- 2) Water availability: We tested this hypothesis using water deficit (WD), annual precipitation (AP) and precipitation of the wettest month (PWM) as measures of water availability. WD, AP and PWM are available at <http://www.worldclim.org/> (Hijmans *et al.*, 2005).

- 3) Water–energy balance: We tested this hypothesis using actual evapotranspiration (AET), a common surrogate of the joint availability of water and energy in the environment (Hawkins & Porter, 2003; Rodríguez *et al.*, 2005). AET is available at <http://www.cgiar-csi.org/data/global-high-resolution-soil-water-balance> (Trabucco & Zomer, 2010). This dataset provides high-resolution global raster climate data related to evapotranspiration processes and rainfall deficit for potential vegetative growth.
- 4) Productivity: We used two metrics as indicators of the rate at which plants produce useful chemical energy, including net primary productivity (NPP), and the normalized difference vegetation index (NDVI). The NPP represents the net amount of solar energy converted to plant organic matter through photosynthesis. The higher the higher the vegetation cover, the higher the NPP. The NDVI represents the aggregate of greenness over the year period and thus is often chosen as the standard proxy for annual biomass productivity. The NDVI is translated to net primary productivity (NPP) by correlation with MODIS data to provide a measure of land degradation and improvement open to economic analysis. Therefore, the NDVI is a measure of photosynthetically active green biomass. The higher the NDVI score, the more living green biomass (natural vegetation and crops if any) can be found. NPP data are available at <http://sedac.ciesin.columbia.edu/data/collection/hanpp> (Imhoff *et al.*, 2004). Data on NDVI (1981-2003) are available at the Food and Agriculture Organization (FAO) of the United Nations (2008), <http://www.fao.org/geonetwork/srv/en/metadata.show?id=37060>.
- 5) Habitat heterogeneity: SSA exhibits several terrestrial habitats and two mountains chains (i.e. the Andes Range and the Coastal Range) that run all along its length. Therefore, we used values generated from the topographic position index (TPI), the habitat diversity index (HDI) and the average elevation (EL) to account for the environmental heterogeneity of this region. The TPI is an algorithm increasingly used to measure topographic slope positions and to automate landform classifications. Data on TPI were extracted from the GLC 2000 (Bartolomé & Belward, 2005) available at <http://www.gvm.jrc.it/glc2000>. The HDI represents the diversity of vegetation structure within each 1° latitudinal band using a modified version of the Shannon-Weiner diversity index (for further details, please refer to the study of Sanciango *et al.*, 2013). Finally, the EL represents the average elevation per each 1° latitudinal band calculated with ArcGIS 10.2 (ESRI, 2014). The TPI, the HDI and the EL were calculated using Land

Facet Corridor Designer in ArcGIS (Jenness *et al.*, 2011) and a digital elevation model (DEM) based on the HydroSHEDS database (Lehner *et al.*, 2008).

- 6) Climatic variability: We used precipitation seasonality (PS), temperature seasonality (TS), and Isothermality (ISO) to test whether climatically less variable areas supports more testate amoeba species diversity. PS, TS and ISO are available at <http://www.worldclim.org/> (Hijmans *et al.*, 2005).
- 7) Species–area: We used the average area of each 1° latitudinal band to assess this hypothesis in SSA. Area values were calculated using the module XTools Pro 8 implemented in ArcGIS.
- 8) Mid–domain effect (MDE): To examine whether testate amoeba species distributions depend just on hard limits (i.e. boundary constraints) imposed by the physical limits of southwestern South America (i.e. the domain); we used the Monte Carlo simulation program, Mid–Domain Null (McCain, 2004). This program simulates species-richness curves based on empirical range sizes or range midpoints within a bounded domain based on the analytical–stochastic models of Colwell & Hurtt (1994). The program is designed to sample with and/or without replacement from the empirical data. When sampling without replacement, the empirical values for range size or midpoints in the simulation are constrained to actual empirical species distributions. Instead, sampling with replacement allows a set of species to be ‘created’, which can be quite different from the original species complement. Sampling without replacement is a randomization technique, whereas sampling with replacement is a bootstrap method (see McCain, 2004 and references cited therein). Here, the observed species richness for each 1° latitudinal band was compared with null–model predictions using 50000 Monte Carlo simulations sampled without replacement from empirical range sizes, to calculate the amplitude of the 95% confidence simulation prediction curves. A high fit between the empirical and predicted curves of species richness is considered as evidence of MDE. Please, see McCain (2004) for a detailed explanation on advantages and disadvantages of sampling with or without replacement.

Principal component analysis

We performed a principal component analysis (PCA) of species distribution data with fitted environmental variables in order to show an additional and independent line of evidence supporting the second prediction of this study (Fig. S1 and Table S2).

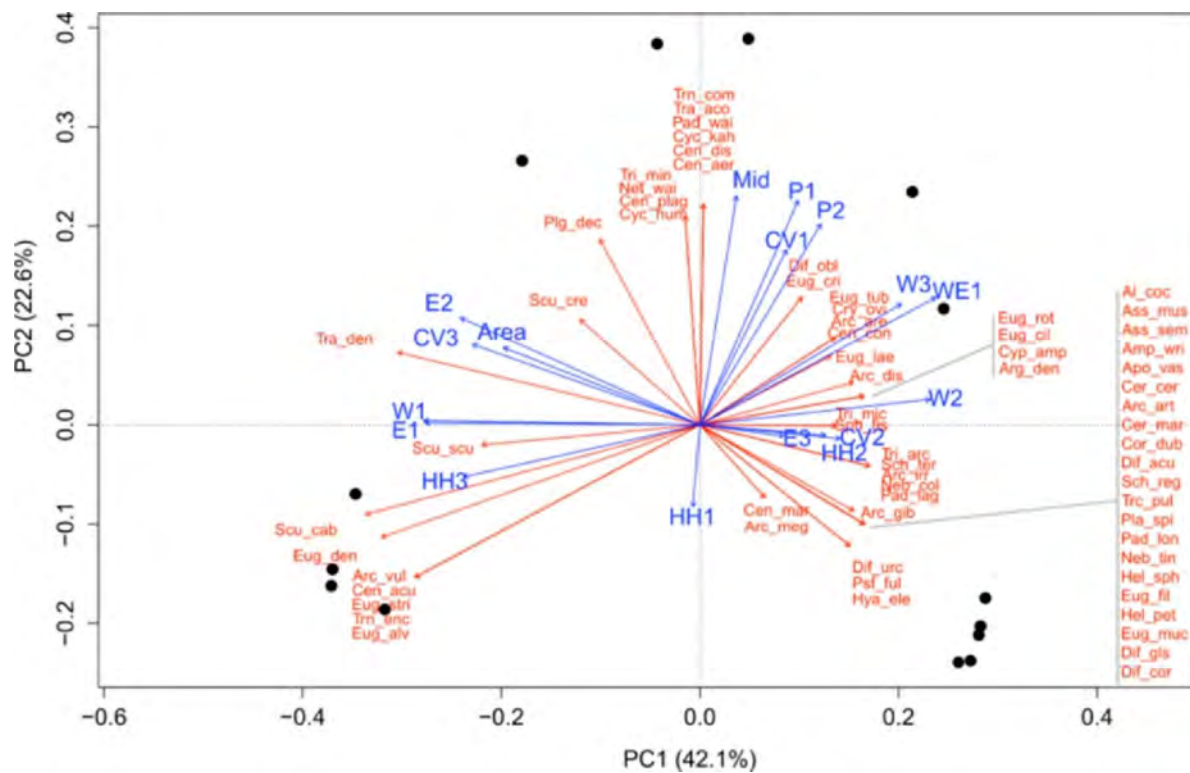


Fig S1 Simplified ordination triplot. Species are represented by red arrows, environmental variables by blue arrows and sites by black dots. Species distribution data were Hellinger-transformed and environmental variables were scaled ([0,1]) prior to analysis. Species distributions were first analysed using a PCA. Environmental variables were then fitted using the function *envfit* of the R package *vegan* (Oksanen *et al.*, 2012). The percentage of variance explained by axes 1 and 2 is shown in brackets. Species with a low contribution to axes 1 or 2 (i.e. located close to the origin) were removed to improve the readability of the figure.

Table S2 Ordination summary statistics. Environmental variables goodness of fit (shown as uncorrected r-squared coefficients) and associated p-values obtained through permutation test.

	R^2	P-value
E1	0.944	0.001
W1	0.937	0.001
WE1	0.888	0.001
E2	0.852	0.001
P1	0.743	0.001
HH3	0.726	0.001
CV3	0.721	0.001
W3	0.683	0.001
P2	0.681	0.001
W2	0.665	0.001
Mid	0.665	0.001
Area	0.551	0.001
CV1	0.473	0.001
HH2	0.246	0.004
CV2	0.197	0.018
E3	0.089	0.191
HH1	0.083	0.216

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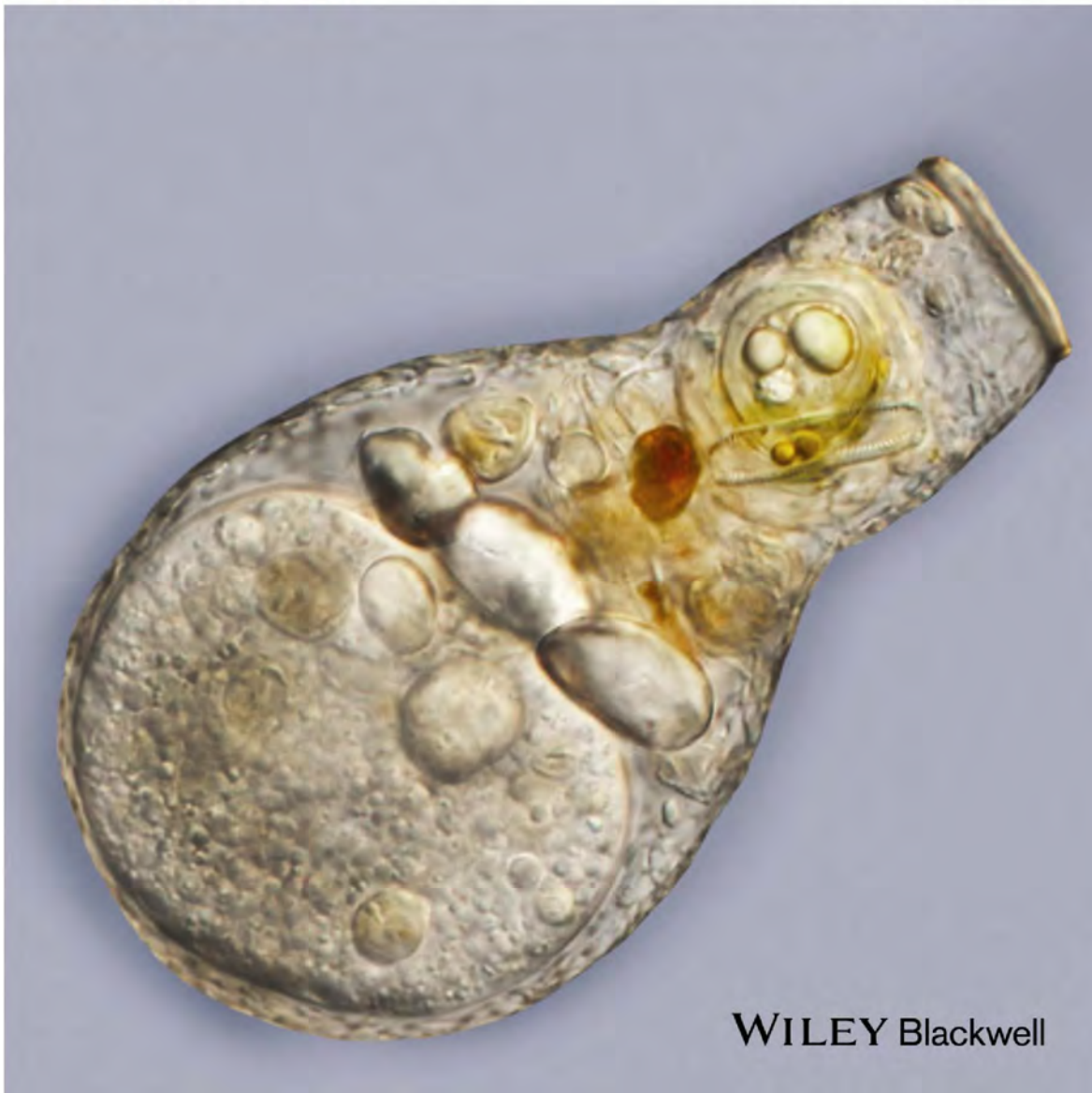
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Chapter 4: Elevational diversity patterns in free-living soil unicellular eukaryotes are driven by evolutionary constraints to water and energy availability

Elevational diversity patterns in free-living soil unicellular eukaryotes are driven by evolutionary constraints to water and energy availability

Leonardo D. Fernández^{1,2}, Christophe V. W. Seppey¹, David Singer¹, Bertrand Fournier¹, Dylan Tatti³, Edward A. D. Mitchell^{1,4} and Enrique Lara¹

¹Laboratory of Soil Biology, Institute of Biology, University of Neuchâtel, Rue Emile Argand 11, CH-2000 Neuchâtel, Switzerland; ²Laboratorio de Ecología Evolutiva y Filoinformática, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario s/n, Casilla 160-C, Concepción, Chile; ³Laboratory of Functional Ecology, Institute of Biology, University of Neuchâtel, Rue Emile Argand 11, CH-2000 Neuchâtel, Switzerland; ⁴Botanical Garden of Neuchâtel, Chemin du Perthuis-du-Sault 58, CH-2000 Neuchâtel, Switzerland

Abstract

Modern soil protists likely evolved under the tropical-like climate that characterized much of the Mesozoic. This fact, coupled with the high diversity and performance that they exhibit in terrestrial warm-humid biomes, suggests that the need for mild temperatures and high humidity is a phylogenetically conserved trait in these microorganisms. Herein, we hypothesized that the strength of this conservatism drives the distribution of soil protist diversity by imposing ecophysiological constraints that limit their capacity to colonize cold sites. To test this hypothesis, we investigated the patterns of elevational variation in diversity, energy-diversity relationship, geographical range sizes and phylogenetic structure in Bacillariophyta, Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida in the Swiss Jura Mountains. All protists exhibited a decline in diversity and an increase in the size of their geographical ranges towards higher and colder elevations. Except for Bacillariophyta, all protist communities were composed of phylogenetically closely related taxa. Finally, phylogenetic structure among communities (phylobetadiversity) was promoted by high levels of turnover. These results suggest that these soil protists lack the necessary ecophysiological adaptations to survive in exceedingly cold environments.

Probably, the retention of their ancestral adaptation to tropical-like conditions imposes strong constraints on their ability to colonize sites with cold abiotic conditions and drives their current spatial patterns in diversity.

Keywords: biogeography, community composition; elevational diversity gradient; high-throughput sequencing; Rapoport effect, soil protists.

Introduction

One of the most conspicuous and best documented spatial patterns of biodiversity is the elevational diversity gradient, which typically describes the variation in species richness along mountainsides (Gaston and Blackburn, 2000). The elevational diversity pattern may exhibit different shapes depending on the scale, taxon studied and local climatic condition to which the mountainside is subjected (Grytnes and McCain, 2007). Most elevational diversity gradients can be broadly classified as decreasing (richness peaks at low elevations), unimodal (richness peaks at mid-elevations) and low plateau with a mid-peak (LPMP, richness peaks at low-mid elevations) (Rahbek, 1995; McCain, 2009, 2010). Less often, species richness varies without following any obvious trend with elevation (Grytnes *et al.*, 2006; McCain, 2009; Taylor *et al.*, 2015).

Recently, there has been growing appreciation for the idea that spatial patterns of biodiversity (i.e. biogeographical patterns), such as the elevational diversity gradient, result from a combination of ecological (e.g. niche-based processes), historical (e.g. climatic stability) and evolutionary (e.g. spatial variation in diversification rates) processes, which together influence spatial variation in speciation, extinction and dispersal rates (Wiens and Donoghue, 2004; Mittelbach *et al.*, 2007). Although not equivalent, historical and evolutionary processes highlight the role that long-term dynamics play on the formation of biodiversity patterns. Therefore, they are often jointly referred to as long-term processes (Rivadeneira *et al.*, 2008; Hortal *et al.*, 2011).

One holistic, parsimonious and growingly accepted hypothesis that integrates ecological and long-term processes to explain biodiversity patterns is the phylogenetic niche conservatism hypothesis. This hypothesis proposes that taxa tend to retain their ecophysiological traits over evolutionary time, and that this trend underlies the biodiversity patterns that we observe today in nature (Wiens and Donoghue, 2004; Wiens *et al.*, 2010). This hypothesis offers several testable predictions that, together, can shed light on the relative role played by ecological and long-term processes in shaping biodiversity patterns (Wiens *et al.*, 2010; Hortal *et al.*, 2011). Several of these predictions are actually derived from biogeographical hypotheses that once were independently proposed to explain biodiversity patterns, and that nowadays, can be linked under the context of the phylogenetic niche conservatism hypothesis. Considering that most modern groups and species originated during the time when the planet was predominately under tropical environments (Behrensmeyer *et al.*, 1992; Graham, 1999), the most basic prediction that the phylogenetic niche conservatism makes is that species richness of current taxa will be higher in sites with the warmest and wettest conditions, because these sites exhibit the environmental conditions in which they originated (Wiens and Donoghue, 2004). This and other predictions are summarized in Table 1 under the context of elevational gradients

These predictions have been extensively tested in plants and animals, proving useful to assess the relative role that ecological and long-term processes play in shaping their biodiversity patterns along different spatial gradients, including elevational gradients (Hawkins *et al.*, 2006; McCain, 2009; Hortal *et al.*, 2011; Qian *et al.*, 2013). By contrast, these predictions are just starting to be investigated in unicellular organisms, including bacteria (Bryant *et al.*, 2008) and testate amoebae (Fernández *et al.*, 2016); and therefore, many of the biogeographical generalizations that have been proposed for plants and animals have not yet been tested in microorganisms. Accordingly, it is still not clear whether biogeographical patterns in microbial diversity are produced and maintained by the same processes proposed for plants and animals. This is a serious gap in knowledge since microorganisms are nowadays considered as the most diverse and abundant organisms on Earth (Whitman *et al.*, 1998; Chao *et al.*, 2006; de Vargas *et al.*, 2015) and play pivotal roles in biogeochemical cycles of all ecosystems (Adl and Gupta, 2006; Massana, 2011).

In recent years, several studies have contributed to improve our understanding on the patterns and mechanisms that drive microbial diversity at broad spatial scales (e.g.,

Bryant *et al.*, 2008; Fernández *et al.*, 2016; Fournier *et al.*, 2016; Lara *et al.*, 2016; Schiaffino *et al.*, 2016; Wang *et al.*, 2016). This trend has been partially driven by the development and application of DNA-based methods, such as high-throughput sequencing techniques (e.g. Illumina-based technologies), which allow the retrieval of hundreds of thousands microbial barcodes per sample and comprehensive analysis of diversity and composition of microbial communities in the environment (Lepère *et al.*, 2013; Logares *et al.*, 2014; de Vargas *et al.*, 2015; Singer *et al.*, 2016). However, most research on microbial diversity has been conducted on prokaryotes or on aquatic microbial eukaryotes, while free-living soil eukaryotic microorganisms, with the exception of fungi (soil protists for short) remained comparatively poorly studied (Foissner, 1999; Chao *et al.*, 2006; Bates *et al.*, 2013; Fernández *et al.*, 2016; Fournier *et al.*, 2016; Lara *et al.*, 2016). Presumably, this trend is driven by the smaller size of the global scientific community specialized in the study of soil protists.

Recent research suggests that, just like several plants and animals (Hawkins *et al.*, 2006; McCain, 2009; Hortal *et al.*, 2011; Qian *et al.*, 2013), soil protists retain their ecophysiological traits over evolutionary time. Particularly, it seems that their need for high humidity and warm temperatures is well-rooted in their evolutionary history and drives their current spatial distribution. While the origin of the first eukaryotic microorganisms probably dates back to the Proterozoic (Porter and Knoll, 2000; Porter *et al.*, 2003; Berney and Pawlowski, 2006; Parfrey *et al.*, 2011), fossil evidence available (Poinar *et al.*, 1993; Schönborn *et al.*, 1999; Ascaso *et al.*, 2005; Sims *et al.*, 2005; Hardwood *et al.*, 2007; Martín-González *et al.*, 2008) and time estimates from molecular clock studies (Pawlowski, 2006; Parfrey *et al.*, 2011; Lahr *et al.*, 2015; Duckert, 2016) suggest that many extant soil protist groups (e.g. euglyphid testate amoebae, ciliates, chlorophytes, diatoms), evolved under the warm and humid climates that characterized much of the Mesozoic (Behrensmeyer *et al.*, 1992). Moreover, soil protists are very diverse in biomes with high yearly rainfall and warm temperatures, such as temperate and tropical rain forests (Foissner, 1997; Bamforth, 2010; Bates *et al.*, 2013; Fernández *et al.*, 2015; Lara *et al.*, 2016), but comparatively less diverse and prevalent in biomes with water deficit and extreme temperatures such as hot (Robinson, 2002; Fernández, 2015) and cold (Smith, 1978; Zapata and Matamala, 1987) deserts. Indeed, soil protists are very sensitive to changes in humidity and temperature (Foissner, 1997; Geisen *et al.*, 2014; Bates *et al.*, 2013). Experimental evidence also reveals that many protists show a higher performance (e.g. increased survival, growth and reproductive rate) at temperatures between ca. 18

and 24 °C (Laminger, 1978; Laybourn and Whymant, 1980; Foissner, 1987; Rose and Caron, 2007; Souffreau *et al.*, 2010; Li *et al.*, 2011). Many of them (e.g. difflugids and some ciliates), simply cannot survive or reproduce without water (Foissner, 1997; Meisterfeld, 2002a; Souffreau *et al.*, 2010). Taken together, these facts suggest that most eukaryotic microorganisms lack the necessary ecological and physiological adaptations to survive in exceedingly cold and hot environments. Probably, the retention of their ancestral adaptation to tropical-like conditions imposes strong constraints on their ability to colonize sites with cold or hot abiotic conditions and drives their current spatial patterns in diversity.

Herein, we aimed to study the patterns and causes of elevational diversity in soil protists along an elevational gradient of beech forests in Switzerland, characterized by having a high humidity and comparable physicochemical properties (i.e. within the range of requirements of the mesic tree species *Fagus sylvatica*), but decreasing temperature with elevation (i.e. covering the whole temperature range of this species). For this task, we first applied a next-generation sequencing approach (Illumina sequencing targeting the v9 region of the SSU rRNA gene) to investigate the variation in richness of five monophyletic groups of soil unicellular eukaryotes over the gradient, including Bacillariophyta (diatoms), Cercomonadida (cercomonadids), Ciliophora (ciliates), Euglyphida (euglyphid testate amoebae), Kinetoplastida (kinetoplastids) and a group combining all of these taxa (referred here as the “soil protist group”). These groups were chosen as models because (1) they are frequent and diverse in soils (2) they have different life histories and functional roles, and (3) are well distributed across the eukaryotic tree. Our rationale behind that choice was to be able to generalize conclusions to as many soil eukaryotes as possible. Secondly, we tested the predictions of the phylogenetic niche conservatism hypothesis summarized in Table 1 to assess the relative role of ecological and long-term processes in shaping their elevational diversity patterns.

Materials and methods

Study site and sampling

Between September and October 2012, we sampled soil protist communities in beech (*Fagus sylvatica* L) forests distributed between 400 and 1300 m a.s.l., in the Jura and Plateau biogeographical regions of Switzerland (Table 2). These forests are unevenly

distributed over the elevational gradient and are largely regarded as being among the few natural beech-dominated forests remaining in Europe (Heiri *et al.*, 2009). The highest sampling forest site was located at the maximum elevation at which these forests occur, close to the top of the Jura Mountains. The whole study area exhibits a maritime temperate climate with an average annual precipitation and temperature of 1200 mm and 8.3 °C, respectively (Heiri *et al.*, 2009). Given that temperature decreases by an average of approximately 0.6 °C for each 100 m increase in elevation (Barry, 2008), it can be estimated that there is an average difference of approximately 5.1 °C between the lowest (warmest) and highest (coldest) forest site. At each forest, we randomly collected three soil cores at a depth and diameter of 5 cm. The content of the three cores was then pooled together to have a representative soil sample of the sampling site. DNA was extracted in the laboratory immediately after sampling.

Along with sample collection, we recorded plant species composition and from this inferred the average ecological values for each sampling site using Landolt codes (Landolt *et al.*, 1977, 2010; Table 2). Landolt's indicator values comprise a model of bioindication that allow determining a set of soil environmental variables based on the plant species present in a site. Landolt's indicator values apply a five-point scale for each of nine variables including: continentality, humus type, light, moisture, moisture variability, nutrients, soil aeration, soil reaction (pH) and temperature. Landolt's values are often preferable over soil environmental measurements made at a particular time and specific point because they integrate temporal and spatial variations in soil environmental conditions (Diekmann, 2003).

DNA extraction, amplification and sequencing

Environmental DNA was extracted using the MoBio PowerSoil DNA isolation kit (Carlsbad, CA, USA) following the manufacturer's instructions. PCR protocols targeting the SSU rRNA gene V9 region of eukaryotes were done according to Amaral-Zetler *et al.* (2009). Sequencing was performed with the Illumina HiSeq 2000 technology to obtain paired-end reads (2 x 200 bp) (Fasteris, Geneva, Switzerland). Quality check (Phred score filtering, elimination of reads without perfect forward and reverse primers, and chimera removal) of the sequences were performed following de Vargas *et al.* (2015). Sequences were grouped into operational taxonomic units (OTUs) using the clustering algorithm swarm v1.2.5 (Mahé *et al.*, 2014). The curated and updated PR² (Guillou *et al.*, 2013) and SILVA

(Pruesse *et al.*, 2007) databases were used to taxonomically assign the OTUs after a selection of the V9 regions according to the primers used for the sequencing. A detailed description of this procedure is provided in the Supplementary Material S1. OTUs were considered as undetermined if the percentage of identity did not reach 80%. One sequence was randomly chosen to represent each OTU. The representative OTUs were used to build a phylogenetic tree using a maximum likelihood approach and the RAxML V.7.7.1 algorithm (Stamatakis *et al.*, 2008) using the GTR+G+I model. Model parameters were directly estimated in RAxML. We also randomly subsampled 50 000 sequences correctly assigned to different soil protists groups from each site to account for unequal sample sizes between microbial communities. From these subsamples, we only selected those assigned to Bacillariophyta, Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida. We performed species accumulation curves for each group using the package vegan (Oksanen *et al.*, 2015) implemented in R 3.1.2 (R Development Core Team, 2011), to assess whether our sampling effort was good enough to record a reasonable proportion of the total OTU richness found at each forest site.

Elevational diversity gradient

We defined a community as all OTUs originating from a single beech forest sample. The taxonomic richness within each community was calculated as the total number of OTUs within each community. Phylogenetic alpha diversity within each community was calculated using Faith's index (Faith, 1992) with the R package Picante (Kembel *et al.*, 2010). Both measures of alpha diversity were significantly correlated (Supplementary Figure S1), and therefore, we only used taxon richness (the observed richness datasets hereafter) in subsequent analyses. The observed richness datasets were plotted against the elevation and the shape of the elevational diversity gradients observed were classified into decreasing or LPMP, following McCain (2007, 2009, 2010).

In the study site, beech forests exhibit an uneven distribution that could bias our interpretation of the elevational diversity patterns. Literature indicates that the shape of the elevational diversity gradient may also vary depending on whether a nonstandardised or standardised dataset is used (Rahbek, 1995; McCain, 2009). Therefore, we re-assessed the shape of the elevational diversity gradient, but this time accounting for sampling biases. To this end, we standardised the observed richness (= nonstandardised richness) datasets for sampling completeness using a combination of range interpolation and

richness estimates. Range interpolation consisted in dividing the entire elevational gradient into 100-m elevational bands and assuming that taxa were present at all elevations between the lowest and highest observed elevations (i.e. taxa had continuous elevational ranges; McCain, 2009). Richness estimations involved the calculation of the expected richness for each 100-m elevational band using a coverage-based richness estimator (Chao *et al.*, 2014). This method estimates richness based on a standardized measure of sampling completeness, allowing a less biased comparison of richness datasets. By contrast, traditional rarefaction approaches estimate richness based on communities of equal size, which systematically biases the degree of differences between community richness (Chao *et al.*, 2014). Richness estimates and their 95% confidence intervals were computed for sample coverage up to doubling the sampling effort invested in the best sampled 100-m elevational band (500 m, three forest sites) using the package iNEXT (Hsieh *et al.*, 2014). The combination of range interpolation and richness estimates resulted in richness datasets standardized for sampling completeness (i.e. the standardised richness datasets). The standardised richness datasets were then used to re-assess the shape of the elevational diversity gradients.

Assessment of the predictions

The hypotheses and predictions are summarized in Table 1. These predictions assume that the ancestral adaptation of soil protists to warm, humid climates imposes a strong constraint on their ability to colonize cold (and hot) sites. These predictions are valid for elevational gradients situated in temperate latitudes. These gradients exhibit wet conditions between their limits and decreasing temperature with elevation (Barry, 2008). In Switzerland, between the plateau and the Jura Mountains, precipitation varies between ca. 1 135 and 1 813 mm, while temperature ranges between ca. 10 and 4 °C (Derungs, 2007).

Species-energy hypothesis: it is expected that soil protist diversity will decrease with elevation (Prediction 1.1.1) and show a positive relationship with temperature (a species-energy effect; Currie, 1991; Prediction 1.1.2). Both predictions were tested constructing linear and quadratic models with the observed richness datasets as response and elevation and environmental variables (as measured by Landolt's indicator values) as descriptors (we only retained temperature, light, humidity, pH and humus content to avoid multicollinearity). The overall model significance and the significance of each descriptor was tested using ANOVA in the R package *vegan*. Next, we re-assessed the predictions of

this biogeographical hypothesis, but this time using the standardised richness datasets to account for sampling biases. Both predictions were re-assessed constructing linear and quadratic models with the standardised richness dataset as response and elevation and the average annual temperature as descriptors. The average annual temperature was calculated based on the average annual temperature reported for the lowest sampling site (10 °C, Swiss Federal Statistical Department, 2016) and assuming a moist adiabatic lapse rate of 0.6 °C for each 100-m increase in elevation (Barry, 2008).

Niche-based processes: community assembly mediated by biotic interactions results in communities of distantly related taxa (phylogenetic overdispersion), whereas community assembly via habitat filtering results in communities of closely related taxa (phylogenetic clustering) (Webb *et al.*, 2002; Bryant *et al.*, 2008). It is therefore predicted that higher sites with colder temperatures will have communities of closely related taxa, because habitat filtering will gain relevance. By contrast, low and warm elevations will have communities of distantly related taxa because optimal and stable thermal conditions will promote niche differentiation via competitive exclusion (Prediction 2.1.1). Alternatively, if the gradient only has optimal temperatures, then low and high elevations will have communities composed of distantly related taxa (Prediction 2.2.1). Finally, if the entire gradient has suboptimal temperatures, then habitat filtering will be the main structuring force of soil protist communities (Prediction 2.3.1). These predictions were tested using mean pairwise distance (MPD) and mean nearest taxon distance (MNTD) indices against a null model comprised of 99 999 randomized trees with taxa shuffling, leading to standardised metrics (ses.MPD and ses.MNTD, corresponding to -NRI and -NTI, respectively) (Kembel *et al.*, 2010). Within a community, -NRI represents the mean phylogenetic distance among all pairs of taxa. Moreover, -NTI represents the mean distance between each taxon and its closest relative. Negative values of these indices indicate phylogenetic clustering, whereas positive values indicate phylogenetic overdispersion. These indices and their statistical significance were computed using the R package picante.

Rapoport effect: it is expected that that decreasing temperature with elevation will progressively filter (select) soil protists with broader ranges and thermal tolerances (generalists), giving rise to a macroecological pattern known as the (elevational) Rapoport effect (Steven, 1989; Stevens, 1992). The Rapoport effect (Prediction 3.1.1) was tested using Pearson's correlations in R to assess the relationship between the mean elevational

range size of all OTUs co-occurring in the same forest site and elevation. A positive relationship between both variables will support the existence of a Rapoport effect.

Beta diversity: two antithetic and non-mutually exclusive basic patterns cause variation in taxa composition (i.e. beta diversity) along environmental gradients, namely taxa replacement or “turnover” (i.e. the loss of a species followed by the gain of another among sites) and differences in taxa richness or “nestedness” (i.e. poorer communities are a gradual subset of the richer ones) (Baselga, 2012; Ulrich and Almeida-Neto 2012). Elevational gradients often exhibit abrupt changes in environmental conditions. Therefore, it is expected that variation in taxa composition will be primarily driven by turnover and secondarily by nestedness, i.e. there will be a strong taxa replacement followed by a loss of taxa among sites due to the incapacity of taxa to colonize increasingly harsh environmental conditions (Prediction 4.1.1). Alternatively, if changes in environmental conditions are gradual, variation in taxa composition will be primarily driven by nestedness, i.e. there will be a gradual loss of taxa with elevation due to a progressive change in abiotic harshness, giving rise to an ecological pattern known as source-sink dynamics (Prediction 4.2.1). Beta diversity was measured using the PhyloSor index (Bryant *et al.*, 2008), and then decomposed into its two additive measures of dissimilarity components (turnover or and nestedness) following Leprieur *et al.* (2012), to estimate the relative contribution of turnover and nestedness in generating beta diversity. Beta diversity computations were conducted with the R package betapart (Baselga and Orme, 2012). The observed values of PhyloSor and its additive measures of dissimilarity were compared to null values calculated from 50 000 random communities ($P < 0.05$) constructed using an independent swap algorithm employing the R packages betapart and vegan.

Mid-domain effect

Geometric constraints have been proposed as a non-deterministic mechanism underlying the elevational diversity gradients along mountainsides (Grytnes and McCain, 2007; McCain, 2009). This hypothesis proposes that obvious or unobvious dispersal barriers situated at both ends of spatial gradients determine the size and the placement of taxa ranges along the elevational space. These barriers are supposed to constrain taxa ranges to the limits of the elevational domain studied, producing both a random overlap of taxa distribution and a peak of richness in the centre of the mountainside (the so-called mid-domain effect; Colwell and Hurtt, 1994). Therefore, the geometric constraint hypothesis

may provide insights of the relative importance of stochastic processes to community assembly along mountainsides: in the absence of deterministic processes, all the elevational variation in diversity is due to geometric constraints. The geometric constraint hypothesis was tested using the Mid-Domain Null program (McCain, 2004) to compute the upper and lower unimodal diversity curves predicted by a mid-domain null model. Null model predictions were constructed using 50 000 Monte Carlo simulations sampled without replacement from empirical taxa-range-size distributions (McCain, 2004). Recording a strong overlap between the empirical diversity and the null model predictions, as well as a positive correlation between the empirical diversity and the average null model predictions supports the occurrence of an elevational mid-domain effect.

Results and discussion

Surveys of beech forests yielded a total of 47 394 sequences distributed in a total of 1 413 OTUs (i.e. here called the soil protist group). In general, the heterotrophic protists Cercomonadida (12 437 sequences, 373 OTUs) and Ciliophora (15 599 sequences, 582 OTUs) were the most abundant and diverse soil protists at each forest site, followed by the also heterotrophic groups Kinetoplastida (10 377 sequences, 223 OTUs) and Euglyphida (8 058 sequences, 157 OTUs), and lastly by the phototrophic group Bacillariophyta (923 sequences, 78 OTUs) (Supplementary Figure S2). Species accumulation curves calculated for each group reached a plateau, indicating that sampling and sequencing covering were good (Supplementary Figure S3). Our results are consistent with other studies; Cercomonadida and Ciliophora often are among the most diverse and abundant heterotrophic soil protists in forests (Foissner, 1997; Bates *et al.*, 2013; Geisen *et al.*, 2015). Euglyphida (Lara *et al.*, 2016) and Kinetoplastida (Vickerman, 2000) are also diverse and common in forest soils, although never at the same level as Cercomonadida and Ciliophora. Bacillariophyta are also considered as a common component of soil protist communities in forests (Sims *et al.*, 2005). However, they appear to be more diverse and abundant in open habitats (e.g. meadows) than in forests, where the highest amount of light reaching the soil surface is lower (Seppey *et al.*, unpublished).

Elevational diversity gradient

In agreement with other studies on elevational diversity gradients (e.g. Rahbek, 1995; McCain, 2009), our analyses revealed that different conclusions can be drawn from non-standardised and standardised richness datasets. The observed (nonstandardised) richness datasets revealed the existence of three elevational diversity patterns (LPMP, decreasing and no pattern), while the standardised richness datasets revealed the existence of two of them (LPMP and decreasing) (Table 3; Supplementary Figure S4). Despite of these differences, both dataset types showed that alpha diversity roughly peaks at low or low-mid elevations before decreasing towards higher and lower elevations. While, the decrease in richness towards higher elevations may be explained by the increasing environmental harshness, e.g. colder temperatures (Stevens, 1992; Grytnes *et al.*, 2006; Grytnes and McCain, 2007), the apparent and incipient decline in richness towards lower elevations may be reflecting the increasing water deficit that characterizes the lowlands of temperate mountains (Barry, 2008). Therefore, if we had extended our sampling towards lower elevations, then we probably would have observed a unimodal diversity pattern for almost all taxa.

The elevational diversity patterns observed corresponded to observations from previous studies reporting that soil protist richness peaks at low or low-mid elevations (decreasing pattern) (Krashevskaya *et al.*, 2007; Heger *et al.*, 2015) or shows no elevational pattern (Mitchell *et al.*, 2004; Shen *et al.*, 2014). However, this is the first time that the LPMP pattern (a widespread elevational trend in richness among reptiles, birds and mammals; reviewed in McCain, 2007, 2009, 2010) is explicitly reported for eukaryotic microorganisms. This pattern may not be unusual among microorganisms. Four out of six groups studied here exhibited this pattern and there is also evidence suggesting that the LPMP has been previously observed but not reported in, for example, testate amoebae (authors' conclusion based on Figure 2 in Mitchell *et al.*, 2004).

Assessment of the predictions

Species-energy hypothesis: Both richness datasets also yielded different outcomes for Predictions 1A and 1B. Except for Cercomonadida, no observed richness dataset showed a statistically significant relationship with elevation (Table 3; Supplementary Figure S4). The lack of statistical relationship between almost all the observed richness datasets and elevation may be explained by an unequal sampling over the gradient. This bias led to a

discontinuous representation of soil microbial communities, which probably undermined the statistical power of the analyses and the chances of detecting a true (statistical) pattern. This problem could have been solved by sampling at regular intervals (e.g., every 100-m increase in elevation), but this was impractical in our study site. These forests exhibit a patchy and uneven distribution over the gradient. Under such a situation, data standardisation is the best way to overcome undersampling issues and detecting patterns (Rahbek, 1995, 2005; McCain 2009). Indeed, the standardised richness datasets showed negative and significant relationships with elevation (Table 3; Supplementary Figure S4).

The observed richness datasets showed no statistical relationship with Landolt's indicator values ($P > 0.05$). By contrast, the standardised richness datasets showed a positive correlation with temperature (Table 3; Supplementary Figure S5). This suggests that the elevational patterns of soil protist diversity are driven by their thermal constraints. This idea is consistent with evidence showing that most protists have a low performance (decreased survival, growth and reproductive rate) (Laminger, 1978; Laybourn and Whymant, 1980; Foissner, 1987; Rose *et al.*, 2007; Souffreau *et al.*, 2010; Li *et al.*, 2011) and diversity (Heger *et al.*, 2015; Fernández *et al.*, 2016; Lara *et al.*, 2016) in sites with cold environmental conditions.

Niche-based processes: The assessment of the phylogenetic structure within communities showed that the soil protist group exhibited both local communities of distantly and closely related taxa (i.e. phylogenetic overdispersion and clustering, respectively), indicating that overall soil protist communities are structured by both biotic and habitat filtering (Figure 1). This outcome provided mixed support to our predictions: low elevations only showed phylogenetically clustered communities, middle elevations both phylogenetically clustered and overdispersed communities, and high elevations only phylogenetically clustered communities. Overall, these results suggested that habitat filtering is important at low and high elevations, while both habitat filtering and biotic interactions shape soil protist communities at mid-elevations. Moreover, we obtained different results after investigating the phylogenetic structure into more specific groups. Bacillariophyta were characterised by phylogenetically overdispersed communities along the entire elevational gradient studied, supporting Prediction 2B; while Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida only exhibited phylogenetically clustered communities, supporting Prediction 2C (Figure 1).

Phototrophic protists, such as diatoms, have higher reproductive rates than heterotrophic protists at cold temperatures (Rose and Caron, 2007). Therefore, diatom growth is less constrained by decreasing temperature than other soil protists. Rather, diatom distribution may be limited by light availability; in forest, sun spotlights are rare and unevenly distributed. Competition for suitable micro-environments must be, therefore, even stronger than for other groups. This must result into competitive exclusion of close relatives and phylogenetically overdispersed communities (Webb *et al.*, 2002). Alternatively, this outcome may be the result of the joint influence of competition at low, warm elevations and ecological facilitation (i.e. a taxon facilitates the occurrence of another in a suboptimal habitat) at cold, high elevations. At least it has been shown that this is the mechanism behind the occurrence of distantly related species of vascular plants over mountainsides (Callaway *et al.*, 2002; Bryant *et al.*, 2008). While, to our knowledge there are no studies on facilitative interaction between soil diatoms, the empirical observation of this interacting biotic factor between estuarine benthic diatoms (Vanelislander *et al.*, 2009) does provide support to this idea.

Phylogenetically clustered communities generally occur in suboptimal sites, where habitat filter plays a major role in community assembly (Webb *et al.*, 2002). This suggests that the entire elevational gradient studied exhibits severe environmental conditions for the phagotrophic Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida. In particular, as winter frost occurs at all elevations along the gradient, it may be possible that the cold and suboptimal temperatures prevailing for much of the year in the study sites (Heiri *et al.*, 2009) constantly filter (select) a subset of cold-tolerant taxa from a regional species pool, leading to the assembly of phylogenetically clustered communities (Bryant *et al.*, 2008; Hortal *et al.*, 2011; Qian *et al.*, 2013). This does not mean that other environmental factors do not play an important role in the assembly of their communities, but rather that the importance of their thermal adaptations on their distribution may minimize that of other factors.

Rapoport effect: In strong agreement with our previous results, geographical ranges of all taxa increased with elevation, conforming to the macroecological pattern of the Rapoport effect (Figure 2; Supplementary Table S1). The observation of this elevational Rapoport effect supported Prediction 3A and suggests that the gradual decrease in temperature progressively filter taxa with broader cold tolerance towards high elevations. Although not universal, the elevational Rapoport effect has been widely

recorded among macroorganisms, proving useful for discriminating between optimal and sub-optimal elevational sites for organisms (Stevens, 1992; Beketov, 2009; Ah-Peng *et al.*, 2012). To our knowledge, the Rapoport effect had only been explicitly tested and demonstrated in latitudinal gradients for eukaryotic microorganisms (Fernández *et al.*, 2016); and therefore, this is the first time ever that an elevational Rapoport effect is demonstrated for microorganisms in general.

Beta diversity: Beta diversity analyses revealed that there is a high and significant dissimilarity in taxa composition between any pair of soil protist communities taken at random from our study site (PhyloSor values ranged from 0.57 to 0.66, $P < 0.05$; Table 4). Also, in all cases beta diversity was mainly due to turnover (PhyloSor_{Turn} values ranged from 0.5 to 0.60, $P < 0.05$) and to lesser extent to nestedness (i.e. PhyloSor_{PD} values ranged from 0.04 to 0.09, $P < 0.05$; Table 4). These results support Prediction 4A and revealed that an important portion of taxa are replaced among forest sites (there is a “true turnover” among sites; Baselga, 2012), while a comparatively low proportion of taxa co-occur among forest sites. These high values of turnover suggested that different elevations are colonized by organisms with different thermal tolerances. Therefore, each forest site may have a unique and probably little explored microbial eukaryote community.

Mid-domain effect: We did not find support for a mid-domain effect in the study site. There was a low overlap between the geometric constraint null model predictions and empirical richness values, as well as inconsistency between the empirical peak of richness and the peak of richness predicted. Also, there was a lack of correlation between the empirical diversity and the average null model predictions (Figure 3; Supplementary Table S2). This finding confirmed that stochastic processes did not play an important role in shaping the elevational diversity pattern and in the assembly of local microbial communities, which is in agreement with similar studies performed on microorganisms at similar spatial scales (Bryant *et al.*, 2008; Charvet *et al.*, 2014).

Conclusions

Our results support the idea that the need for warm, humid environmental conditions represents an evolutionary constraint on the spatial distribution of soil protist diversity. The use of next-generation sequencing allowed a comprehensive analysis of microbial diversity

and composition over the gradient. The different groups of protists exhibited elevational diversity patterns that varied in shape depending on the taxon studied. Despite these differences, our analyses showed that all taxa exhibited a decrease in richness towards higher and colder elevations. This trend was only significant when richness was standardized, except with Cercomonadida where this pattern was also explicit when data were not standardised. Standardising the observed richness was required because the sampling sites were unevenly distributed over the elevational gradient. These facts invite to re-assess the elevational diversity gradient in the microbial taxa that apparently do not exhibit a significant relationship with elevation or environmental variables (e.g. temperature and water availability). In addition, all soil protist groups exhibited a combination of elevational variation in phylogenetic structure, range sizes and taxa composition that suggest that they have thermal constraints that limit their ability to colonize cold sites at high elevations. Most likely these thermal constraints also drive the spatial distribution of their diversity over other spatial gradients (e.g. see Fernández *et al.*, 2016; Lara *et al.*, 2016). Future studies should aim at re-assessing the generality of our findings in mountains present in contrasted climatic regions (e.g. cold or arid mountains), along continuous and more extensive elevational gradients (e.g. from sea level to the mountaintops), and using much more detailed information on the environmental factors that potentially drive the distribution of these microorganisms in soils.

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Table 1 Predictions that can be drawn from the phylogenetic niche conservatism hypothesis, which proposes that climatic conditions within which a taxon evolved –warm and humid conditions for most modern taxa– should be conserved in the present (Wiens and Donoghue, 2004). These predictions stem from biogeographical hypotheses that once were independently proposed to explain biodiversity patterns and are valid for elevational gradients situated at temperate latitudes, which exhibit little variation in precipitation and decreasing temperature with elevation (Barry, 2008).

	<i>Biogeographical hypothesis</i>	<i>Theory</i>	<i>Testable prediction</i>
1	Species-energy (Currie, 1991; Evans <i>et al.</i> , 2005)	1.1. Richness positively related to temperature rather than to any other environmental factor	1.1.1. Richness decreases with elevation, showing a decreasing or LPMP diversity gradient 1.1.2. Richness positively related to temperature
2	Niche-based processes (Webb <i>et al.</i> , 2002; Bryant <i>et al.</i> , 2008)	2.1. Local community assembly mediated by competition at low, warmer elevations and habitat filtering at high, colder elevations 2.2. Local community assembly mediated by competition along the entire elevational gradient (gradient with benign temperatures) 2.3. Local community assembly mediated by habitat filtering along the entire elevational gradient (gradient with harsh temperatures)	2.1.1. Communities of distantly and closely related taxa at low and high elevations, respectively 2.2.1. Communities of distantly related taxa along the gradient 2.3.1. Communities of closely related taxa along the gradient
3	Rapoport effect (Steven, 1989; Stevens, 1992)	3.1. Temperature decreases with elevation and progressively filters (selects) taxa with broader elevational ranges and wider thermal tolerances (generalists)	3.1.1. Positive correlation between elevation and taxa-range-sizes distributions
4	Beta diversity (Hortal <i>et al.</i> , 2011; Baselga, 2012)	4.1. Abrupt changes in temperature with elevation produce sharp changes in beta diversity 4.2. Gradual changes in temperature with elevation produce progressive changes in beta diversity	4.1.1. Beta diversity is primarily produced by turnover 4.2.1. Beta diversity is primarily produced by nestedness (source-sink dynamics)

Table 2 Elevation, coordinates and Landolt's indicator values (five points) estimated for each sampling site.

Site elevation (m a.s.l.)	Latitude (° N)	Longitude (° E)	Landolt's indicator values								
			<i>D</i>	<i>F</i>	<i>H</i>	<i>K</i>	<i>L</i>	<i>N</i>	<i>R</i>	<i>T</i>	<i>W</i>
458	47°08'56.60"	7°35'01.51"	1.74	3.48	3.30	2.48	2.15	3.44	3.00	3.26	2.00
505	47°02'27.23"	7°11'19.57"	2.78	2.94	2.78	2.89	2.44	2.78	3.33	3.28	1.44
548	47°06'47.04"	7°30'16.56"	2.25	3.19	3.50	2.57	1.75	2.88	2.67	3.56	1.50
580	47°21'52.00"	7°06'24.00"	2.24	3.24	3.19	2.55	2.29	3.38	3.05	3.48	1.62
600	47°00'18.20"	7°30'15.52"	2.00	3.25	3.50	2.00	1.50	3.00	3.25	3.63	1.75
772	47°11'01.34"	6°52'16.24"	1.86	3.29	3.43	2.54	2.14	3.71	3.00	3.32	1.71
799	46°34'04.44"	6°19'47.52"	2.59	3.03	3.55	2.55	2.10	2.97	3.21	3.22	1.41
877	47°02'23.39"	6°57'07.32"	2.13	3.25	3.63	2.53	2.06	3.06	3.14	3.22	1.44
1262	46°27'26.83"	6°05'52.09"	2.75	3.09	3.63	2.63	2.19	3.06	2.94	3.03	1.31
1308	47°04'32.25"	6°52'19.19"	2.45	2.93	3.46	2.84	2.75	2.67	3.27	2.91	1.42

D: soil aeration; *F*: moisture; *H*: humus content; *K*: continentality; *L*: light; *N*: nutrients; *R*: soil reaction or pH; *T*: temperature; *W*: moisture variability

Table 3 Relations between (a) nonstandardised richness and elevation, (b) standardised richness and elevation, and (c) standardised richness and average annual temperature. Relationships were modelled with both linear (L) and quadratic (Q) models. The better model was selected using the Akaike's information criterion. The last column (*Pattern*) describes whether the elevational diversity pattern observed in (a) or (b) was classified as decreasing (D), low plateau with a mid-peak (LPMP) or as not showing any pattern (NP).

<i>Relations</i>	<i>Eukaryotic microbial group</i>	<i>Linear model</i>		<i>Quadratic model</i>		<i>Better Model</i>	<i>Pattern</i>
		<i>R</i> ²	<i>P</i>	<i>R</i> ²	<i>P</i>		
(a) Nonstandardised Richness vs elevation	Soil protist group	0.205	0.622	0.256	0.523	L	LPMP
	Bacillariophyta	0.104	0.333	0.101	0.706	L	LPMP
	Cercomonadida	0.513	0.020	0.734	0.047	L	D
	Ciliophora	0.014	0.744	0.021	0.834	Q	NP
	Euglyphida	0.146	0.276	0.316	0.229	L	LPMP
	Kinetoplastida	0.334	0.080	0.360	0.609	Q	LPMP
(b) Standardised Richness vs elevation	Soil protist group	0.699	0.002	0.699	0.353	L	LPMP
	Bacillariophyta	0.886	0.000	0.896	0.222	L	LPMP
	Cercomonadida	0.853	0.000	0.847	0.425	L	D
	Ciliophora	0.579	0.006	0.785	0.021	Q	LPMP
	Euglyphida	0.776	0.000	0.842	0.077	L	LPMP
	Kinetoplastida	0.593	0.006	0.877	0.003	Q	LPMP
(c) Standardised Richness vs average annual temperature	Soil protist group	0.733	0.002	0.766	0.353	L	–
	Bacillariophyta	0.898	0.000	0.919	0.222	L	–
	Cercomonadida	0.869	0.000	0.881	0.425	L	–
	Ciliophora	0.625	0.006	0.833	0.021	Q	–
	Euglyphida	0.801	0.000	0.877	0.077	L	–
	Kinetoplastida	0.638	0.006	0.904	0.003	Q	–

Table 4 Observed and expected (50 000 random communities) values of beta diversity (PhyloSor) and of its two additive measures of dissimilarity, namely turnover (PhyloSor_{Turn}) and nestedness (PhyloSor_{PD}) for the study site.

<i>Taxon</i>	<i>PhyloSor</i>		<i>PhyloSor_{Turn}</i>		<i>PhyloSor_{PD}</i>	
	<i>Observed</i>	<i>Expected</i>	<i>Observed</i>	<i>Expected</i>	<i>Observed</i>	<i>Expected</i>
Soil protist group	0.60	0.45 ^{**}	0.56	0.44 ^{**}	0.04	0.00 ^{***}
Bacillariophyta	0.57	0.32 ^{**}	0.50	0.41 ^{**}	0.07	0.00 ^{***}
Cercomonadida	0.64	0.51 ^{**}	0.60	0.48 ^{**}	0.04	0.00 ^{***}
Ciliophora	0.66	0.55 ^{**}	0.59	0.51 ^{**}	0.07	0.00 ^{***}
Euglyphida	0.61	0.47 ^{***}	0.55	0.42 ^{***}	0.06	0.00 ^{***}
Kinetoplastida	0.61	0.51 ^{***}	0.51	0.44 ^{**}	0.09	0.00 ^{***}

* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

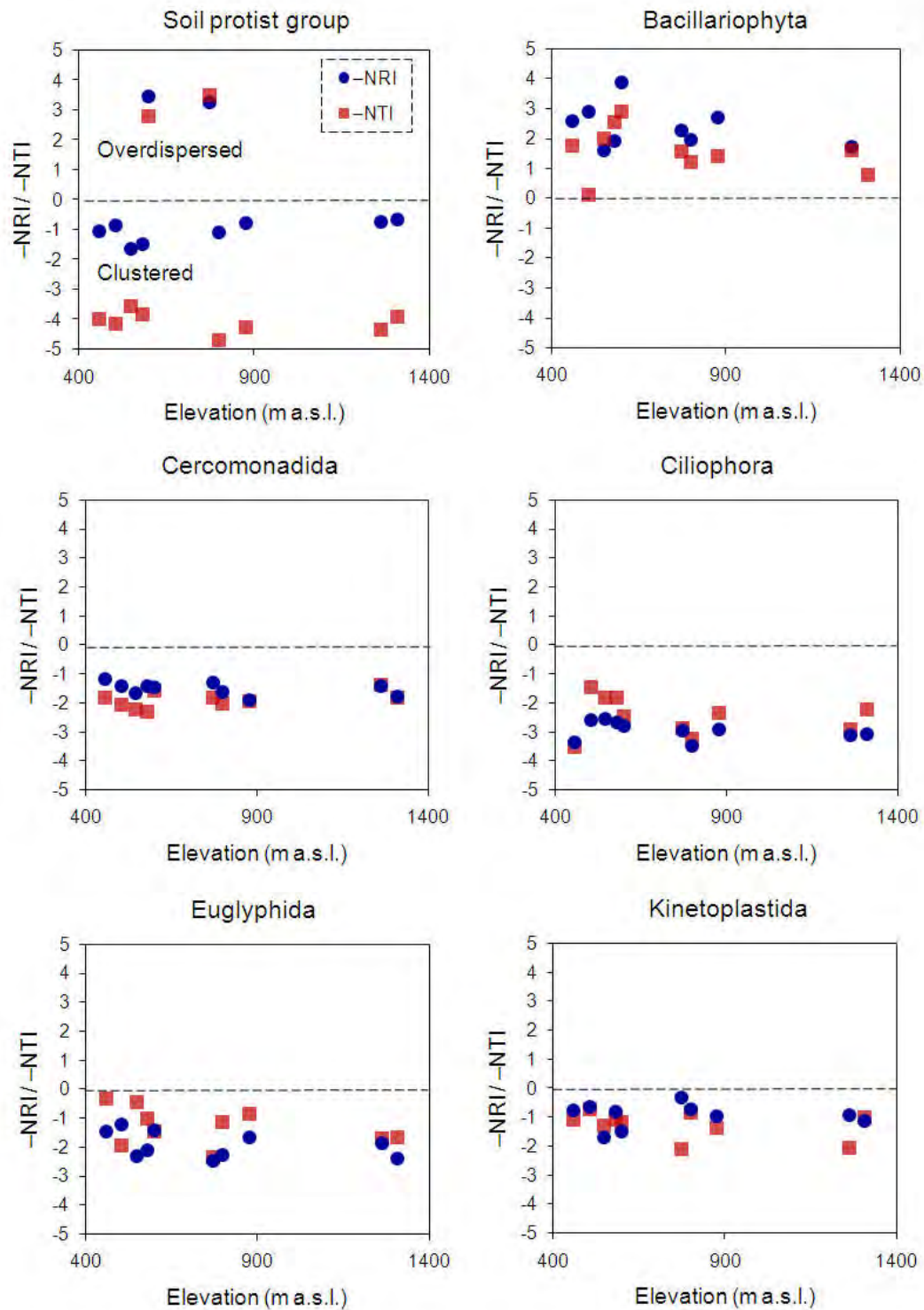


Figure 1 Variation in community phylogenetic relatedness along the gradient studied as measured with both $-NRI$ and $-NTI$ indices. Above zero values indicate phylogenetic overdispersion; while below zero values indicate phylogenetic clustering.

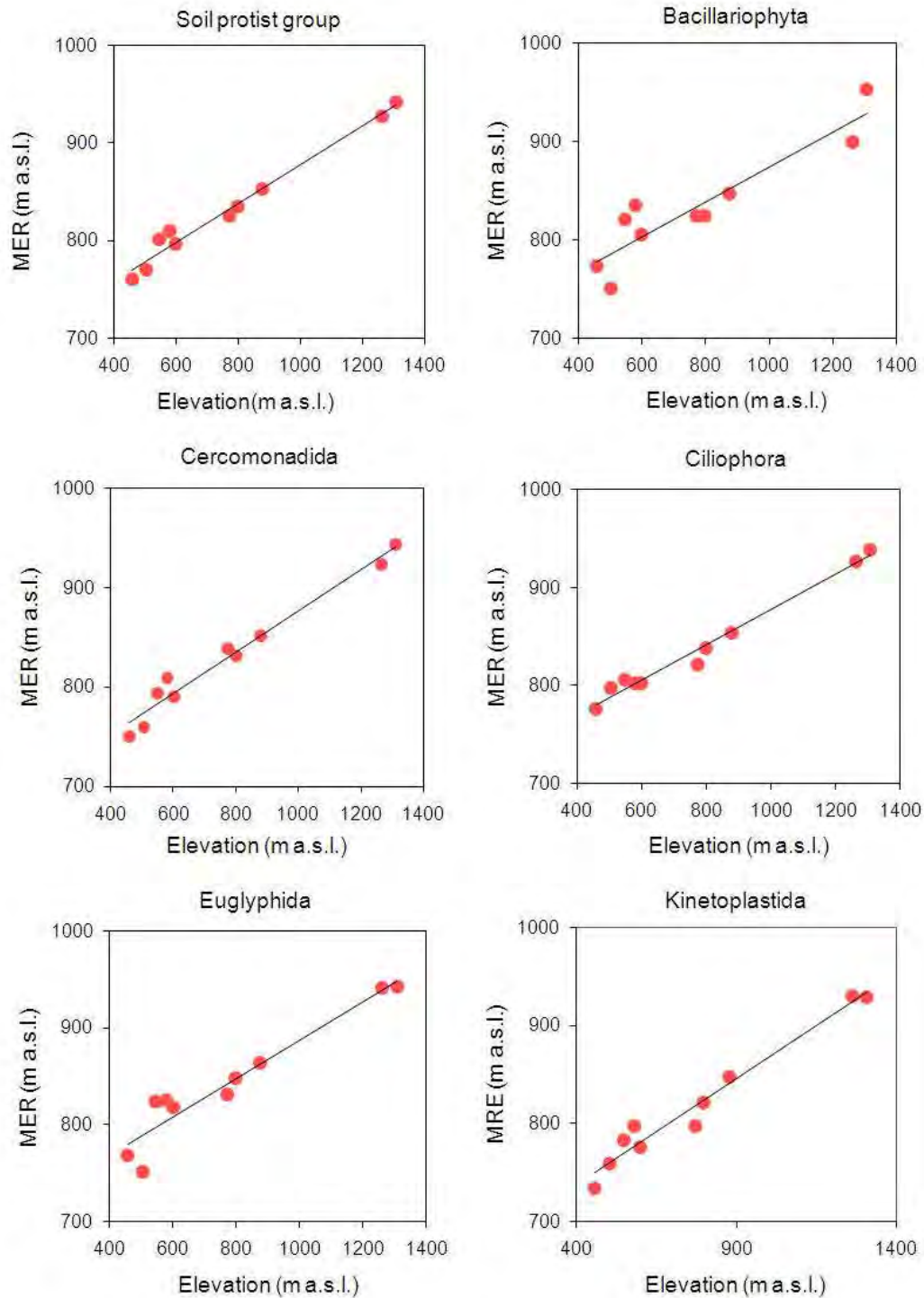


Figure 2 Relationship between the mean elevational range size (MER) of all eukaryotic microbial groups studied and elevation. All eukaryotic microbial groups exhibited a significant ($P < 0.05$, Supplementary Table S1) increase of their elevational ranges with elevation, conforming to the macroecological pattern of the Rapoport effect.

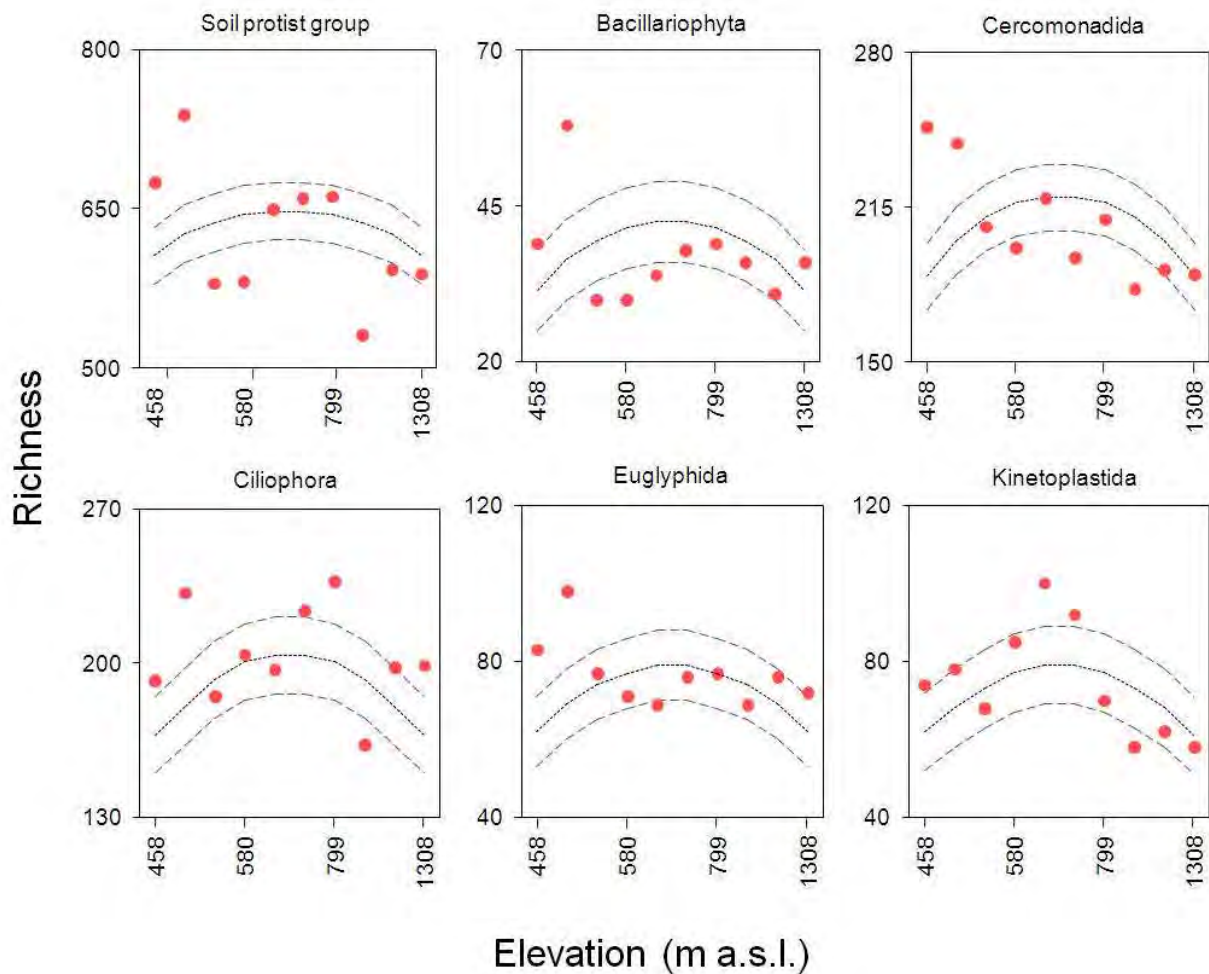


Figure 3 Richness (solid circles), upper and lower 95% confidence limits (broken lines) and average null model predictions (dotted line) computed with the Mid-Domain Null program (50 000 Monte Carlo simulations sampled without replacement from empirical taxa-range-size distributions). In all cases, the observed richness did not show significant relationship ($P > 0.05$) with null model predictions (Supplementary Table S2), indicating that the observed patterns were not significantly influenced by random effects.

Appendix to “Elevational diversity patterns in free-living soil unicellular eukaryotes are driven by evolutionary constraints to water and energy availability”

SUPPLEMENTARY MATERIAL

Elevational diversity patterns in free-living soil unicellular eukaryotes are driven by evolutionary constraints to water and energy availability

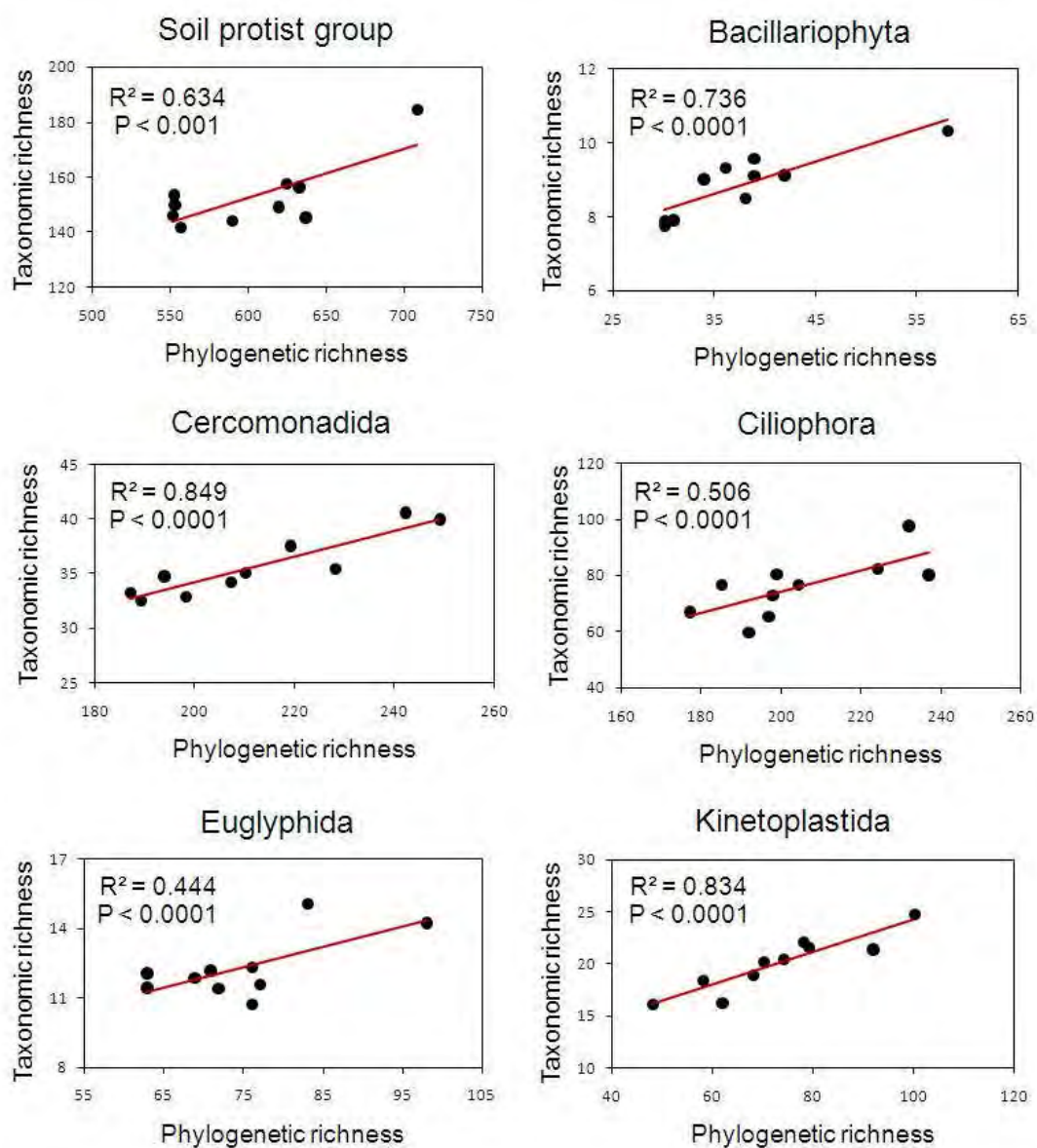
Leonardo D. Fernández, Christophe V. W. Seppey, David Singer, Bertrand Fournier, Dylan Tatti, Edward A. D. Mitchell and Enrique Lara

Supplementary material S1: *DNA extraction, amplification and sequencing*

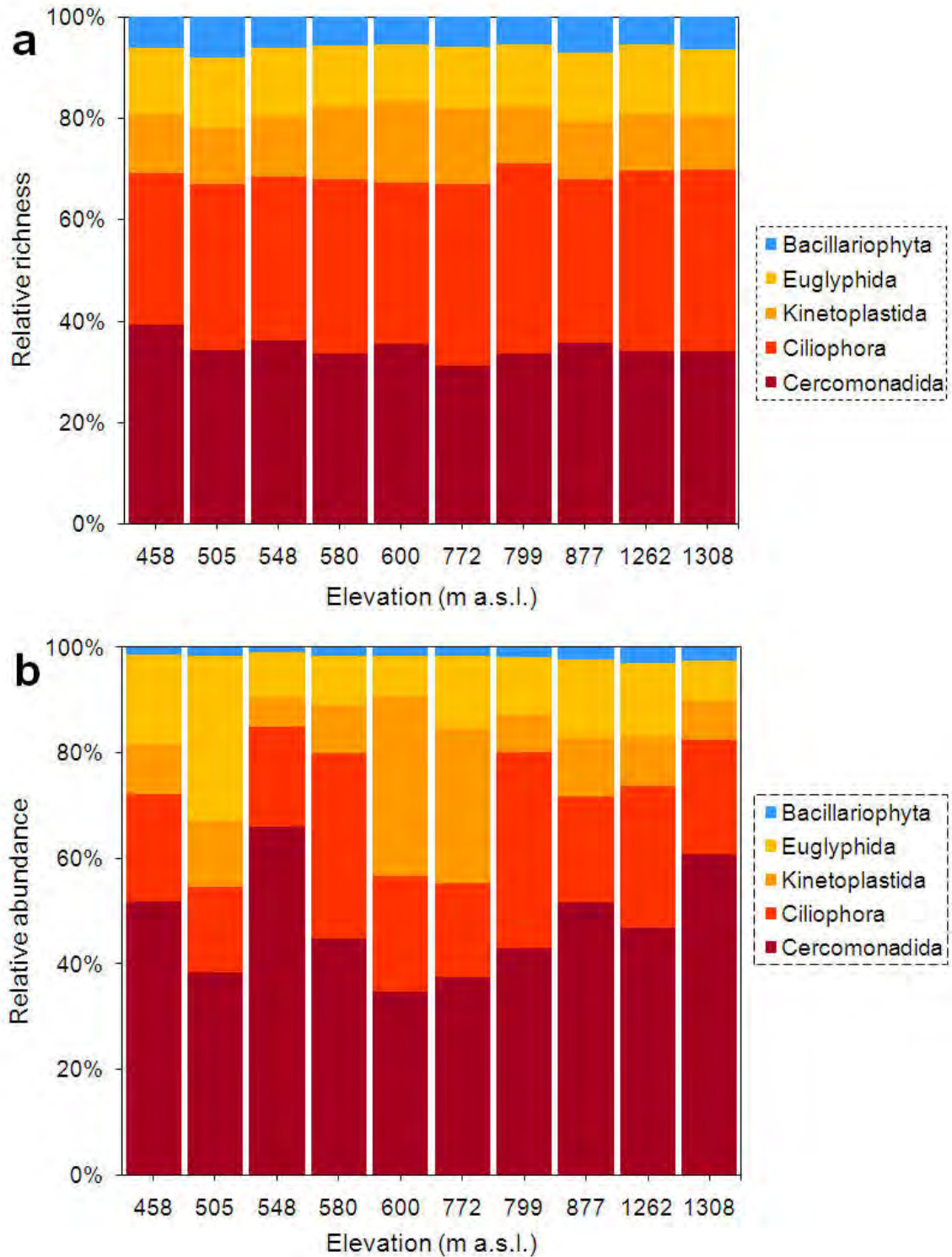
DNA was extracted using MoBio PowerSoil extraction kit (Carlsbad, CA, USA) according to the manufacturer instructions. The SSU rRNA V9 region was amplified using the broad spectrum eukaryotic primers 1380F/1510R (CCCTGCCHTTTGTACACAC/CCTTCYGCAGGTTACCTAC) described in (Amaral-Zettler *et al.*, 2009). PCR reactions were run in triplicates with a PTC-200 Peltier Thermo Cycler (BioConcept, Allswill, Switzerland) with 1ng of environmental DNA, 6µL of 10 x PCR buffer, 0.6µL of each primer, 0.6µL of each dNTP 400µM (Promega, Dübendorf, Switzerland) and 0.2µL of 0.05 U/µL Go Taq (Promega, Dübendorf, Switzerland). The volume was adjusted to 30µL with ultra-pure water. Amplification was conducted with the following conditions: denaturation at 94°C for 3 min, 30 cycles at 94 °C for 30 sec, 57 °C for 60 sec and 72 °C for 90 sec and final extension at 72 °C for 10 min (Amaral-Zettler *et al.*, 2009). PCR products were purified through QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and pooled together at the same concentration prior to sequencing. A DNA library was prepared using the New England Biolabs's kit NEBNext DNA Sample Prep Master Mix Set 1. Illumina HiSeq sequencing was done by Fasteris (Geneva, Switzerland) using an Illumina HiSeq 251 2000 technology to obtain paired-end reads (2X 100bp).

The PR2 database (Guillou *et al.*, 2013) was used as the reference database for a first taxonomic assignation of reads and OTUs; only sequences containing complete forward and reverse primers described above were retained. SSU sequences from bacteria and archaea were also added to the PR2 database from the Silva database (Pruesse *et al.*, 2007) in order to identify and remove eventual prokaryotic sequences from the analysis. The prokaryotic SSU sequences were truncated from the general primer 1389F (TTGTACACACCGCCC) (Amaral-Zettler *et al.*, 2009) to the end of the SSU rRNA

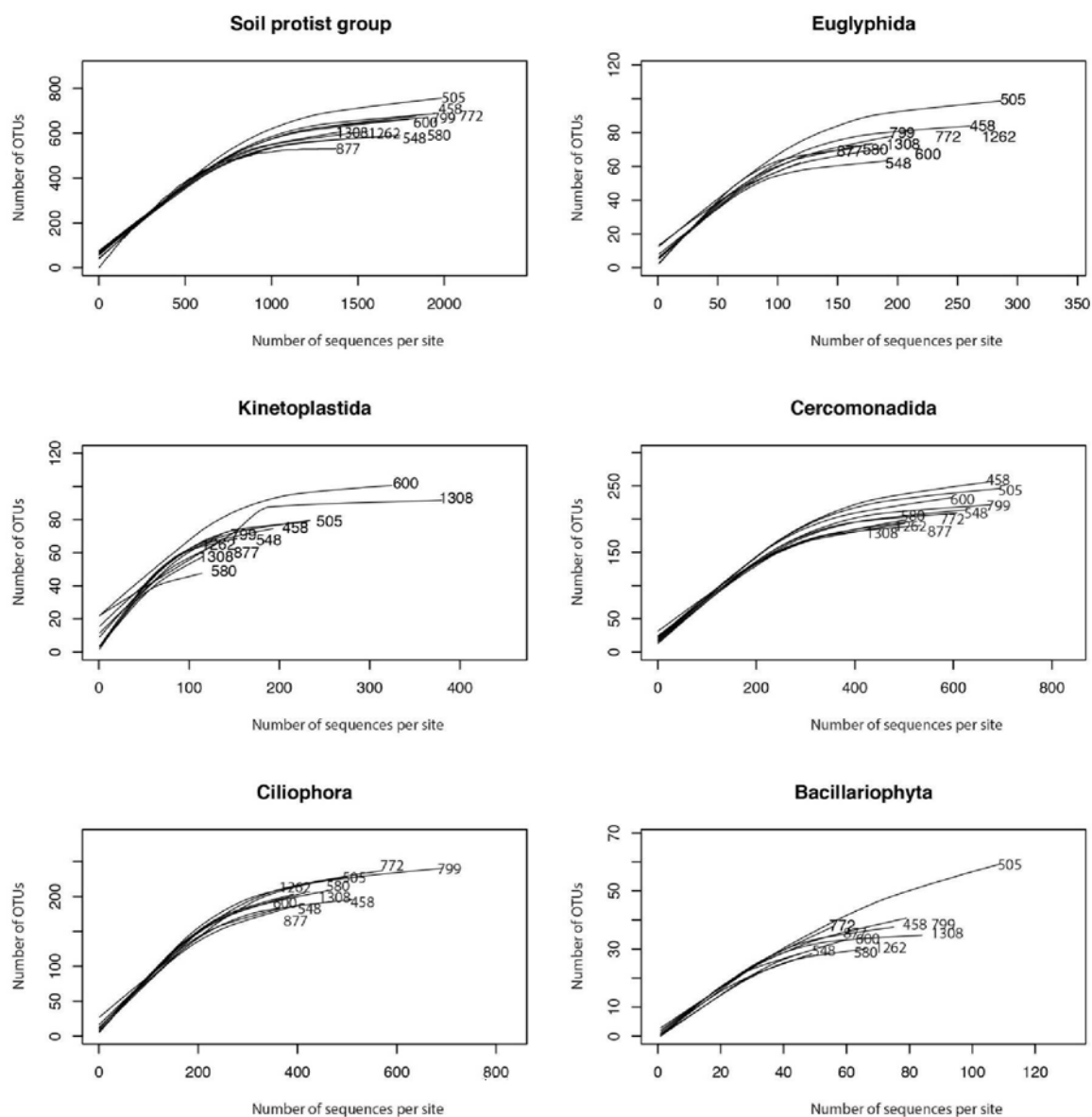
sequence and kept as ortholog of the eukaryotic V9 fragment. The truncated prokaryotic sequences were then de-replicated before being added to the PR2 database. Reads were merged using the program Flash (v1.2.9) (Magoc and Salzberg, 2011) and demultiplexed into samples using the program Sabre (<https://github.com/najoshi/sabre>). Good quality sequences were selected with a custom script inspired from the method used in de Vargas *et al.* (2015). Chimeric sequences were then discarded using the software Usearch (7.0.1090) (Edgar, 2011) by comparing reads against the PR2 (Guillou *et al.*, 2013) and Silva (Pruesse *et al.*, 2007) databases and against reads within the sample. Only sequences containing complete forward and reverse primers described above were retained. In order to remove artifactual sequences, we kept only those that were found at least three times in two samples (de Vargas *et al.*, 2015). OTUs were clustered using the software swarm (v. 1.2.5) (Mahe *et al.*, 2014) with the default set-up. OTUs were then taxonomically assigned by aligning the dominant sequence of every OTU against the PR2 database using ggsearch (Fasta package v. 36.3.6: <http://faculty.virginia.edu/wrpearson/fasta/CURRENT/>). The OTUs were considered as undetermined eukaryotes if their percentage identity with sequences of PR2 was lower than 80% as in (de Vargas *et al.*, 2015). Prokaryotic sequences were sorted out by aligning our data against the Silva database. We removed also sequences belonging to Metazoa or Embryophyceae.



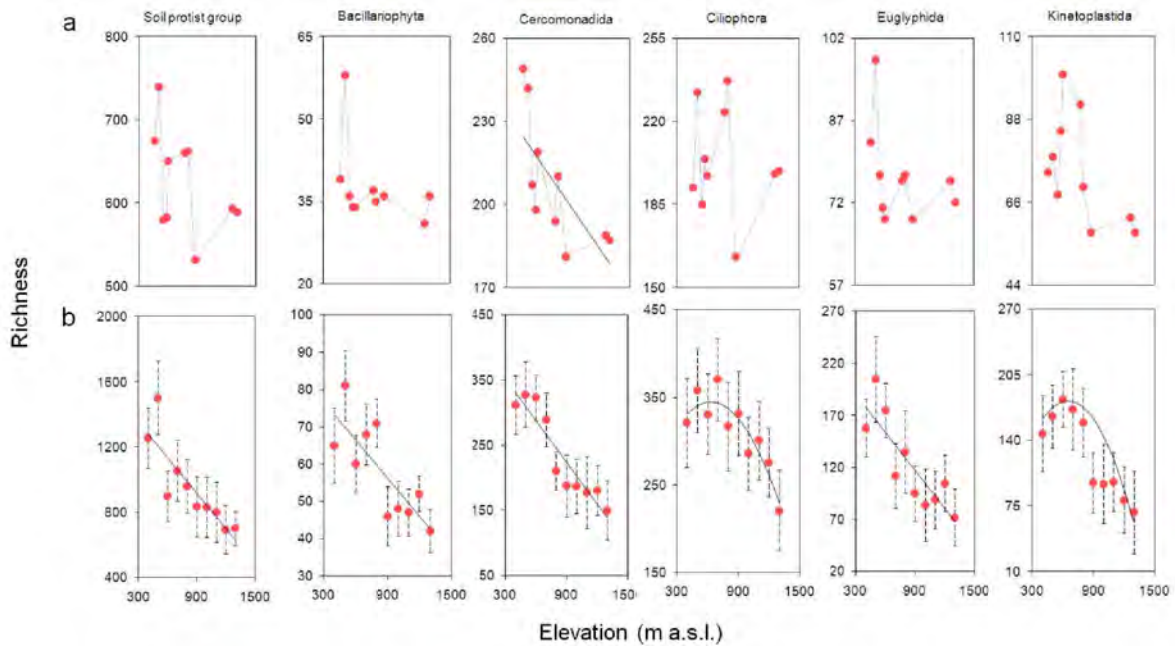
Supplementary Figure S1 Simple linear correlation between the taxonomic richness and phylogenetic richness of each eukaryotic microbial group.



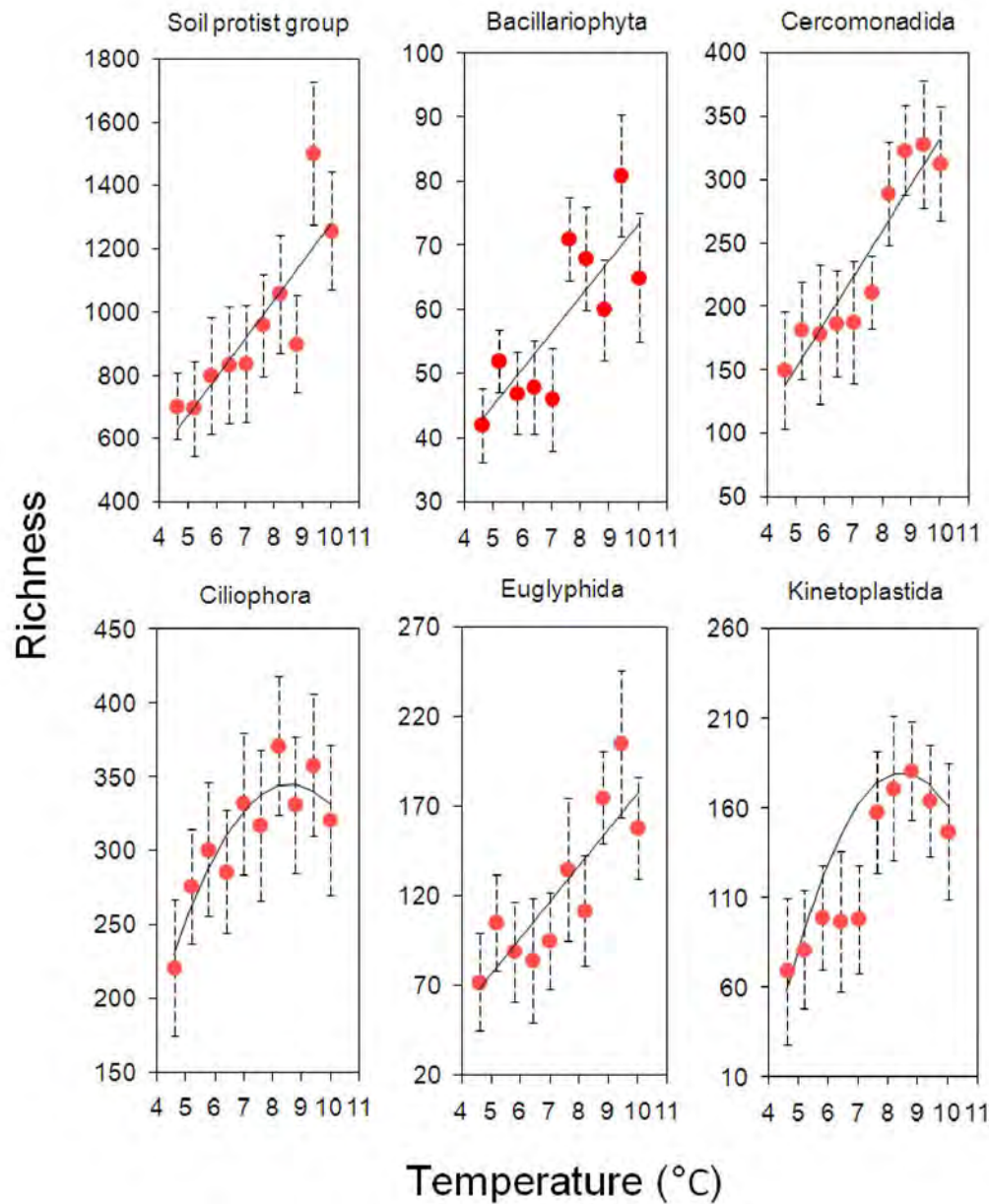
Supplementary Figure S2 Relative richness and abundance recorded for each eukaryotic microbial group studied at each elevational forest site.



Supplementary Figure S3 Species accumulation curves calculated for each eukaryotic microbial group studied. In all cases, species accumulation curves tended to reach a plateau, indicating that sampling and sequencing covering were good at each elevational forest site. The numbers at the end of each line depict site elevations as described in Table 2, within the main body of the manuscript.



Supplementary Figure S4 Relationships between elevation and: (a) the observed (nonstandardised) and (b) the standardised richness of each eukaryotic microbial group. The trends were modelled with linear and quadratic models. The better model was selected based on the lower value of Akaike's information criterion and is shown (when significant) as a solid line. More details about the models can be found in Table 3, within the main body of the manuscript.



Supplementary Figure S5 Relationship between the average elevational changes in temperature and the standardised richness of each eukaryotic microbial group. The trends were modelled with linear and quadratic models. The better model was selected based on the lower value of Akaike's information criterion and is shown as a solid line. More details about the models can be found in Table 3, within the main body of the manuscript.

Supplementary Table S1 Pearson's correlations between the mean elevational range size of all eukaryotic microbial groups studied and elevation (the Rapoport effect)

<i>Eukaryotic microbial group</i>	R^2	P
Soil protist group	0.980	0.000
Bacillariophyta	0.849	0.000
Cercomonadida	0.970	0.000
Ciliophora	0.983	0.000
Euglyphida	0.918	0.000
Kinetoplastida	0.964	0.000

Supplementary Table S2 Pearson's correlations between the empirical diversity and the average null model predictions calculated with a mid-domain null model 50 000 Monte Carlo simulations sampled without replacement from empirical taxa-range-size distributions) implemented in the Mid-Domain Null program (McCain, 2004)

<i>Eukaryotic microbial group</i>	R^2	P
Soil protist group	0.005	0.854
Bacillariophyta	0.049	0.539
Cercomonadida	0.077	0.437
Ciliophora	0.035	0.603
Euglyphida	0.128	0.310
Kinetoplastida	0.380	0.058

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Chapter 5: A lake-dwelling microalga (Bathycoccaceae) exhibits population genetic structure among lacustrine populations due to geographic and ecological isolation

A lake-dwelling microalga (Bathycoccaceae) exhibits population genetic structure among lacustrine populations due to geographic and ecological isolation

Leonardo D. Fernández^{1,2}, Cristián E. Hernández¹, M. Romina Schiaffino³, Irina Izaguirre⁴ and Enrique Lara²

¹Laboratorio de Ecología Evolutiva y Filoinformática, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario s/n, Concepción, Chile; ²Laboratory of Soil Biodiversity, University of Neuchâtel, Rue Emile Argand 11, Neuchâtel, CH-2000, Switzerland; ³Centro de Investigaciones y transferencia del Noroeste de la Provincia de Buenos Aires, CONICET, UNNOBA, Junín, 6000, Argentina; ⁴Departamento de Ecología, Genética y Evolución, IEGEBA (CONICET-UBA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Buenos Aires, C1428EHA, Argentina

Abstract

Microorganisms have huge populations, small sizes and many build dormant stages to resist under environmental conditions. Accordingly, it is assumed that microorganisms have unlimited dispersal and gene flow and do not display population genetic differentiation. We analysed the internal transcribed spacer operon sequences (i.e. the ITS1-5.8S-ITS2 complex) of a lake-dwelling microalga (Bathycoccaceae) to: (a) assess its population genetic structure and differentiation in six lakes of the Argentinean Patagonia, and (b) determine and disentangle the relative role that both isolation by distance and isolation by environment play in shaping the patterns of spatial genetic variation in this microorganism. A haplotype network analysis revealed a few common haplotypes intermixed with several unique haplotypes mainly restricted to the northwestern and southern lakes. Also we observed moderate to high genetic differentiation between lake populations. A spatially explicit Bayesian clustering model grouped lake populations in three distinct genetic unities, confirming that there is population genetic structure. A multiple regression analysis on matrices of genetic, geographic and environmental distances revealed that gene flow between populations is limited due to a combination of

isolation by distance and isolation by environment. Our results therefore led us to reject the hypothesis that there is unlimited gene flow between lake-dwelling microorganisms.

Keywords: freshwater protists, isolation by distance, isolation by environment, landscape genetics, Patagonia

Introduction

Free-living aquatic microorganisms are among the most diverse and ubiquitous living entities on Earth (Logares et al., 2014; de Vargas et al., 2015) and play a pivotal role in biogeochemical cycles (Stockner and Antia, 1986; Massana, 2011). Because of their huge populations, small size and capability to form dormant stages to survive adverse environmental conditions, microorganisms were largely assumed to display unlimited passive dispersal and gene flow among populations (Beijerinck, 1913; Finlay, 2002; Fenchel and Finlay, 2004). While some highly abundant and euryoecious marine microorganisms appear to have unlimited dispersal and a lack of genetic structure among populations (Evans et al., 2005; Šlapeta et al., 2006; Lara et al., 2009; Casteleyn et al., 2010; Van Gremberghe et al., 2011), currently there is a growing body of evidence supporting the idea that many aquatic microorganisms do have limited dispersal and gene flow. Several studies on marine microalgae have reported genetic differentiation among neighbouring populations despite dispersal potential through currents (Ryneckson et al., 2006; Nagai et al., 2007; Godhe and Hårnström, 2010; Hårnström et al., 2011; Sjöqvist et al., 2015). Inland waters have a patchy distribution in the landscape, and moreover, the physicochemical properties of freshwaters differ more between water bodies than between different geographic points within the relatively buffered marine realm (Díaz et al., 2000). Therefore, it can be expected that populations of micro-organisms living in those systems will tend to be more diverse than those living in the sea. Indeed, in inland waters, microorganisms also have complex population genetic structure comparable with macroorganisms (Kim et al., 2004; Evans et al., 2009; Logares et al., 2009; Van den

Wyngaert et al., 2015). Limited gene flow has even been reported among freshwater populations of microorganisms that were separated by less than 10 km (Rengefors et al., 2012).

Although genetic differentiation of microbial organisms into distinct populations has frequently been confirmed, very little is known about how the heterogeneity of landscapes influences the genetic connectivity and structure of microbial populations (Van Gremberghe et al., 2011; Rengefors et al., 2012; Van den Wyngaert et al., 2015). Patterns of genetic differentiation often reflect spatial variation in gene flow. The heterogeneity of landscapes can influence gene flow in two main ways: (1) isolation by distance and (2) isolation by environment (Thorpe et al., 2008; Lee and Mitchell-Olds, 2011). Under a model of isolation by distance (Wright, 1943), geographic distance and barriers restrict gene flow among populations, resulting in a correlation between genetic and geographic distances. Under a model of isolation by environment (Wang and Summers, 2010), gene flow among populations inhabiting different ecological environments is limited by selection against dispersers moving between them or by individual preference to remain in a particular environment (Nosil, 2004; Nosil et al., 2005), resulting in an association between genetic and environmental distances. Isolation by distance has frequently been suggested as a valid model to explain the occurrence of genetic differentiation among certain microbial populations (Casteleyn et al., 2010; Demura et al., 2014). Currents and oceanic basins appear to restrict dispersal and gene flow among marine populations of microorganisms (Casabianca et al., 2012; Godhe et al., 2013). Lack of hydrological connectivity and long-term ice cover has been suggested as important barriers to dispersal and gene flow among lacustrine microbial populations (Rengefors et al., 2012). Isolation by environment, on the other hand, appears to have a large impact on the genetic structuring of microbial populations inhabiting different but highly connected aquatic environments (de Vargas et al., 2002; Ryneerson et al., 2006; H rnstr m et al., 2011).

So far, most of the studies that have assessed the patterns and causes of spatial genetic diversity in populations of aquatic microorganisms have focused on ecosystems of the northern hemisphere (e.g. Ryneerson et al., 2006; Godhe and H rnstr m, 2010; H rnstr m et al., 2011; Van Gremberghe et al. 2011; Casabianca et al., 2012; Lowe et al., 2012; Godhe et al., 2013; Van den Wyngaert et al., 2015); and to our knowledge, there are currently no similar studies on lake-dwelling microorganisms from historically undersampled areas, such as southern Argentinean Patagonia. Because of the distribution

of the landmasses in each hemisphere, basic limnological properties differ completely from Northern Hemisphere counterparts. In this region, lakes represent an interesting playground to assess the influence of actual landscape processes on the genetic structure of microbial populations. These lakes span extensive geographical and environmental gradients, are hydrologically disconnected from each other and located within areas exhibiting potential terrestrial and marine barriers to dispersal, including the Andes and the Magellan Strait. Therefore, different lakes might harbour highly differentiated microbial populations due to limited gene flow.

A recent molecular survey of free-living unicellular eukaryotes in a set of lakes situated in southern Argentinean Patagonia (Schiaffino et al., 2016), revealed the occurrence of a new member of Mamiellophyceae, which was later characterized as being the only known freshwater member of family Bathycoccaceae (Lara et al., unpublished data). This newly erected chlorophyte family (Marin and Melkonian, 2010) comprises the smallest known photosynthetic eukaryote, *Ostreococcus tauri* (Courties et al., 1994). The extreme abundance of these organisms in marine environments (Countway and Caron, 2006) coupled to their small size would suggest that their geographical distributions should tend toward cosmopolitanism (Fenchel and Finlay, 2004). Indeed, such trends have been observed, and genotypes are better separated by different environmental conditions than by distance (Demir-Hilton et al., 2011). However, as suggested before, freshwater environments should promote higher population genetic structure in micro-organisms.

In this study, we tested the hypothesis that there is population genetic structure among lake populations of microalgae. Based on internal transcribed spacer operon sequences (i.e. the ITS1-5.8S-ITS2 complex) of the recently described freshwater Bathycoccaceae we aimed: (a) to assess the population genetic structure and differentiation in this lake-dwelling microalga in six lakes of the Argentinean Patagonia, and (b) to determine and disentangle the relative role that both isolation by distance and by environment play in shaping the patterns of spatial genetic variation in this microorganism.

Materials and methods

Sampling strategy

Water samples were collected from the euphotic zone of six different lakes distributed between the Chubut, Santa Cruz and Tierra del Fuego provinces in the southern Argentinean Patagonia (between latitude 45°55' and 54°36' S) during spring 2007 and 2008. The water bodies sampled included lakes Musters, Posadas, Cardiel, Desierto, San Luis and Yehuin (Figure 1, Table 1). We measured several environmental parameters at each lake (Supplementary Table S1). Temperature, pH, conductivity and dissolved oxygen (DO) were measured *in situ* with portable meters (Horiba D-54 meter, Kyoto, Japan and Hanna HI 9146, Villafranca, Italy). Nutrients (Ammonium N-NH₄, nitrate N-NO₃, nitrite N-NO₂ and phosphate P-PO₄), chlorophyll *a* (chl *a*), dissolved organic and carbon (DOC) were analyzed following the methods described in (Schiaffino et al. 2011). Additional detailed information on the lakes studied can be found in Quirós and Drago (1999).

Molecular methods

Water samples for nucleic acid extractions were pre-filtered *in situ* through a 50 µm net and about 1 litre of water of each lake was filtered sequentially through 20, 3 and 0.2 µm-pore size polycarbonate filters with a vacuum pump. The filters were preserved in cryovials with 1.8 mL of lysis buffer (40 mM EDTA, 50 mM Tris-HCl, 0.75 M sucrose) and stored at –80°C until nucleic acid extraction. The 0.2-3 µm size fraction was used for this study. Nucleic acid extractions were performed with a phenol/chloroform procedure as described in Unrein et al. (2005) and extracts were stored at –80°C until analysis.

We used an alignment comprising SSU and LSU rRNA sequences of several representatives of the Bathycoccaceae/Mamiellophyceae clade, both environmental and derived from cultures. We chose the primers to amplify a region that could encompass the whole ITS operon (i.e. the ITS1-5.8S-ITS2 complex), and to be specific only to this clade and not to amplify Monomastigales, which can be common in freshwaters (Marin and Melkonian 2010). We aimed to use this operon because variation within ITS regions is useful for identification of population groups of eukaryotic microalgae at broad spatial scales (Bakker et al., 1992). The resulting primers were BatSSUF 5'-CCGGGCTTTTTCAAGTCTGGT-3' and BatLSUR 5'-TTGTCTGAACTGAGGTCAAAGG-

3'. We performed a 40 cycles PCR with an annealing temperature of 55 °C using Promega's GoTaq polymerase (Madison, Wisconsin, USA). Products (ca. 2kb) were cloned into apCR2.1 Topo TA cloning vector and transformed into *E. coli* TOP10' One Shot cells (both from Invitrogen) according to the manufacturer's instructions. Clone inserts were amplified using PCR primers. White colonies were picked and PCR was performed directly without any cultivation step of the bacteria. Sequencing was performed on right-sized clones with an ABI PRISM 3700 DNA Analyzer (PE Biosystems, Genève, Switzerland) using a BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems).

Population data analyses

Descriptive statistics of genetic diversity, including the number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π) and number of segregating sites, were estimated for each population using DnaSP v.5 (Librado and Rozas, 2009). A haplotype network was constructed using a Median-Joining algorithm implemented in the program NETWORK 4.5 (<http://www.fluxus-technology.com>) (Bandelt et al., 1999) to determine the genealogical relationship between the haplotypes and the spatial distribution of the haplotypes. Genetic divergence among populations was estimated by calculating the pairwise genetic differentiation (pairwise F_{ST} statistics) in ARLEQUIN v3.5 (Excoffier and Lischer, 2010) based on 50 000 permutations ($P < 0.05$). Sequential Bonferroni corrections were used to adjust significance levels for simultaneous inferential tests (Rice, 1989).

Population genetic structure

The existence of spatial discontinuities (i.e. the existence of genetic structure) among populations was investigated using the program Geneland v.4.0.5 (Guillot et al. 2005a, 2005b; Guillot et al. 2008). Geneland implements a population statistical model with Bayesian inference in a set of georeferenced individuals with sequences data. The goal of this model is to infer and locate the genetic discontinuities between populations of georeferenced genotypes, considering the uncertain localization of the sampled individuals. The number of clusters was determined by running MCMC (Markov chain Monte Carlo) iterations five times, allowing K (i.e., the most probable number of populations) to vary, with the following parameters: 10 000 000 MCMC iterations, maximum rate of the Poisson

process fixed to 100 (this is the default value of the software), the minimum $K=1$, maximum $K=6$ (values that allow exploring a wide potential number of populations, and considering the maximum spatial subdivision in the latitudinal gradient considered). The maximum number of nuclei in the Poisson-Voronoi tessellation was fixed at 300 ($3 \times$ maximum rate was suggested by Guillot et al. 2005b). After inferring the number of populations in the data set from these five runs, the MCMC was run two times with K fixed to the inferred number of clusters, with the other parameters the same as above. The posterior probability of population membership for each pixel of the spatial domain was then computed.

Isolation by distance and isolation by environment

We used multiple regression analysis on matrices of genetic, geographic and environmental distances (MRM; Legendre and Legendre, 1998; Lichstein, 2007), to investigate the extent to which isolation by distance and isolation by environment (either together or separately) influence the genetic connectivity and structure of microbial populations. Unlike other methods for analyzing correlations between distance matrices (e.g. Mantel test; Mantel, 1967); MRM performs a multiple regression analysis between two or more distance matrices, using permutations to determine the significance of the coefficients of determination. MRM was conducted using Slatkin's linearized F_{ST} as response and geographic and environmental distance matrices as predictors. The geographic distance matrix was calculated with the geographic distances between each pair of lakes, using their geographical coordinates and Euclidean distances. To construct the environmental distance matrix, we log transformed environmental variables to avoid skewed trends and then checked for multicollinearity. Next, we normalized all variables to accommodate the different units of the different variables (Clarke et al., 2005) and used these values to build up a distance matrix based on Euclidean distances. MRM analyses were conducted using 50 000 random permutations of the rows and columns of the dependent matrix (while keeping the other matrices fixed) in the package *ecodist* (Goslee and Urban, 2007) implemented in R 3.2.3 (R Development Core Team, 2011). Sequential Bonferroni corrections were used to adjust significance levels for simultaneous inferential tests (Rice, 1989).

Results

We obtained positive PCR products for six lakes located in Patagonia and Tierra del Fuego (Musters, Posadas, Cardiel, Desierto, San Luis and Yehuín) out of a gradient of 14 lakes spanning from Patagonia to the Antarctica (Schiaffino et al., 2016). We cloned the PCR products resulting in a total of 168 ITS sequences (593 bp). For the whole study site, genetic diversity indices revealed a total of 18 haplotypes (h) and 20 segregating sites (S), as well as a total haplotype (H_d) and nucleotide (π) diversity of 0.787 and 0.00364, respectively (Table 1). Genetic diversity indices varied widely among lake populations. Excepted for nucleotide diversity, the southernmost lake population (Yehuín), exhibited the highest values of genetic diversity indices, followed by the northernmost (Musters) and central (Desierto) lake populations, respectively (Table 1). The three other populations (Posadas, Cardiel and San Luis) exhibited comparatively lower values for these indices (Table 1). Overall, the number of haplotypes ranged from 8 to 3, the number of segregating sites between 10 and 2, haplotype diversity between 0.879 and 0.211, and nucleotide diversity between 0.00539 and 0.00037 (Table 1).

The haplotype network suggested that haplotypes have a geographically structured distribution in the Argentinean Patagonia (Figure 2). Haplotype H1 was the most common and was absent only in the southernmost lake (Yehuín). Haplotype H2 was the second most common and was absent from the northernmost lake (Musters). Several other haplotypes were only present at specific lakes. Particularly noteworthy were those present in the central lake Desierto and the southernmost lake Yehuín. Four and six haplotypes were exclusive to these lakes, respectively.

All pairwise F_{ST} values were significant after Bonferroni correction for multiple testing ($\alpha = 0.05$). Genetic divergence between all lake populations was moderate to high, ranging between 0.154 and 0.771 (Table 2). Even geographically close lacustrine populations, such as Yehuín and San Luis, which are ca. 50 km apart, exhibited high genetic divergence (Table 2). The highest genetic divergence was found between the populations of lakes Posadas and San Luis; two demes that are located in areas geographically wide apart (ca. 770 km) (Table 2).

The Geneland analysis inferred three genetic clusters distributed across the north-central, south-central and southernmost zones of the Argentinean Patagonia (Figure 3). These clusters were not necessarily composed of geographically close lake populations. The first cluster was composed of populations occurring in northern (lakes Musters and

Posadas) and central (Cardiel) lakes. The second cluster was composed of populations occurring in central and southern lakes (Desierto and San Luis, respectively). Finally, the third cluster included only the population of the southernmost lake (Yehuín).

MRM analyses revealed significantly positive correlations between the genetic, geographic and environmental distance matrices (Table 3). MRM showed that a high proportion of the genetic differentiation observed between microalga populations is explained by a combination of isolation by distance and isolation by environment. On the other hand, isolation by distance explained much more variation than isolation by environment. The coefficient of determination for geographic distances was about two times greater than the coefficient of determination for environmental distance, suggesting that isolation by distance explained the majority of genetic distance but that isolation by environment also contributed significantly (Table 3).

Discussion

This study provided strong support for the hypothesis that there is population genetic structure among lake populations of freshwater microorganisms. Contrary to what has been predicted under a model of unlimited dispersal and gene flow (Finlay, 2002; Fenchel and Finlay, 2004), we observed moderate to high levels of genetic diversity between populations (Table 1). In addition, we found few common haplotypes intermixed with several unique haplotypes (Figure 2) and moderate to high genetic differentiation among populations (Table 2). These outcomes are clearly inconsistent with the above mentioned model.

The populations of the microalga studied showed a moderate to high levels of genetic divergence, with F_{ST} values ranging between 0.154 and 0.771. In general, F_{ST} values observed were higher than those reported for other genetically structured populations of aquatic eukaryotic microorganisms, including, for instance, lake-dwelling dinoflagellates (F_{ST} values, 0.117-0.376; Rengefors et al., 2012) and brackish water diatoms (F_{ST} values, 0.109-0.286; Ryneerson et al., 2006). It must be noted, however, that the divergence values indicated for dinoflagellates and diatoms were measured on microsatellite data and are therefore not directly comparable to our results. Because of the high F_{ST} values observed, we conclude that there is considerable and significant genetic

differentiation among the populations of the microalga studied. In addition, the spatially explicit Bayesian clustering approach (Geneland) identified three main genetically distinct clusters in the Argentinean Patagonia (Figure 3). One cluster included the populations of lakes Musters, Posadas and Cardiel, a second cluster included the populations of lakes Desierto and San Luis, and a third cluster consisted only of the southernmost population (Lake Yehuín). Taken together, these analyses suggested that there is limited dispersal and gene flow between lake microalga populations studied.

Isolation by distance and isolation by environment

In agreement with other studies on landscape genetics (Ogden and Thorpe 2002; Crispo et al. 2006; Thorpe et al. 2008; Lee-Yaw et al. 2009), our MRM analyses revealed that limited gene flow between lake populations might be the result of both isolation by distance and isolation by environment (Table 3). Recently, a simulation analysis showed that the use of MRM is appropriate to quantify the effects of geographic and ecological isolation when dispersal rates are low or moderate (Wang, 2013). Our analyses of genetic population structure (F_{ST} and Geneland analyses) suggested that our model microorganism exhibits a low to moderate dispersal and gene flow, and therefore we considered our MRM output robust enough to support our conclusions.

The model organism of this study is included within Bathycoccaceae (Lara et al., unpublished data), a family comprising very small microalgae with cells ranging from 0.5 to 2.5 μm in size (Marin and Melkonian, 2010). This microalga inhabits lakes that are influenced by a variety of natural and human dispersal vectors (e.g. migratory water birds and sport fishermen) (Quirós and Drago, 1999) that could potentially contribute to passively disperse their propagules towards distant freshwater bodies. Simulation analyses (Wilkinson et al., 2012) and empirical evidence (Wilkinson, 2001; Yang et al., 2010; Lara et al., 2011) also suggests that smaller microorganisms can be easily airborne dispersed over large spatial scales. From a theoretical point of view, therefore, this microalga should not have any population genetic structure as this would potentially be erased by fast dispersal and high gene flow (Finlay, 2002; Fenchel and Finlay, 2004). In spite of this, our analyses supported geographic isolation as an important contributor to patterns of spatial genetic variation in populations of our model microorganism. This contrasts with observations on marine Bathycoccaceae (*Ostreococcus*, *Bathycoccus*), where intraspecific diversity seems to be driven by local environmental conditions rather

than geographical distance (Demir-Hilton et al., 2011; Simmons et al., 2016). Overall, this suggests that, at least in Bathycoocaceae, living in freshwater favours allopatric genetic differentiation.

Possibly, geographic isolation is an important contributor to patterns of spatial genetic variation in populations of our model microorganism because it does not have the appropriate adaptations to travel over large distances as an active cell or under a cystic stage. Active cells of aquatic eukaryotic microorganisms are potentially vulnerable to dehydration during overland transport (Weisse, 2008); and we do not know if this microalga is capable of forming a resting stage (e.g. a cyst), that could allow individuals to survive and effectively disperse to other lakes under adverse environmental conditions. *Mantoniella*, a genus contained within Mamiellophyceae can form cysts (Ellegaard et al., 2016), suggesting that this could also be the case for some microalgae included within Bathycoocaceae. Notwithstanding this, the viability of cysts varies widely between and within species (Foissner, 2007), and all of them lack adaptations for air dispersal (Foissner, 2008). Therefore, not all cysts necessarily survive under adverse conditions. This might explain why isolation by distance frequently appears as an important contributor to patterns of spatial genetic variation in populations of aquatic microorganisms (Casteleyn et al., 2010; Rengefors et al., 2012; Demura et al., 2014).

Moreover, our analyses demonstrated that environmental isolation also contributed to explain part of the differentiation among lake populations. Patagonian lakes differ significantly in terms of their abiotic characteristics. Lakes Musters, Posadas, Cardiel and San Luis range from mesotrophic to eutrophic, whereas lakes Desierto and Yehuín are classified as ultraoligotrophic or oligotrophic (Quirós and Cuch, 1985; Quirós and Drago, 1999). In addition to exhibiting particular physicochemical conditions (Schiaffino et al., 2011), these lakes also are distributed over an extensive geographical area, so they are subjected to different climates (Paruelo et al., 1998). Consequently, patagonian lakes represent environmentally contrasting islands surrounded by a suboptimal soil matrix that may be potentially limiting dispersal and gene flow among populations inhabiting different lakes via selection against dispersers (Nosil, 2004; Nosil et al., 2005). The outcome obtained using the Geneland program is consistent with this idea: the lakes that harboured the three identified genetic groups (see Figure 3) also exhibit affinities in their physicochemical characteristics (Quirós and Cuch, 1985; Quirós and Drago, 1999; Schiaffino et al., 2011). In agreement with a few previous studies (de Vargas et al., 2002;

Ryner et al., 2006; H rnstr m et al., 2011), we therefore conclude that isolation by environment have an important (and often overlooked) impact on the genetic structuring of microbial populations. This should, however, be further tested over larger scales and using more sites and ideally more taxonomic groups.

An alternative cause of population differentiation

The large genetic differentiation observed between lake populations could also be explained in term of the monopolization hypothesis (De Meester et al., 2002). This hypothesis states that genetic differentiation between populations of lake-dwelling organisms can be explained by rapid population growth and local adaptation after historical founder effect, which result in the effective monopolization of resources (a priority effect). Once a population is locally adapted, the presence of a large resting propagule bank buffer against new immigrants, so enhancing priority effects.

The lakes of north central Patagonia (Musters, Posadas, Cardiel) are much older than the lakes located in the northwest (Desierto) and the south (San Luis, Yehu n). The north central lakes originated during the Mesozoic through tectonic processes (Vilela, 1971; Bellosi, 1995; Gonz lez D  az and Di Tommaso, 2014), while the lakes of the northwest and south originated during the Holocene after the retreat of the ice-sheet at the end of the Last Glacial Maximum (Vilela, 1971; Mariazzi et al., 1987; Quir s et al., 1988). These antecedents and our data suggest that northwestern and southern lake populations descend of ancestral colonizers coming from the north central Patagonia. Overall, haplotype diversity was high but nucleotide diversity was low (Table 1), suggesting recent differentiation with rapid population growth in the Argentinean Patagonia (Grant and Bowen, 1998; Muller, 2008; Palma et al., 2012). Also, the haplotype network (Figure 2), revealed a few common haplotypes intermixed with several unique haplotypes mainly restricted to the northwestern and southern lakes. Haplotype H1 was the most widespread and abundant haplotype in the study site, and therefore, could likely be the ancestral haplotype of this microalga. Besides having many closely related derivatives and a more or less central position in the network, this haplotype was also represented by sequences coming from the oldest, north central lakes. Geneland analyses also identified north central lake populations as being genetically distinct from those present in the northwest and the south, supporting a founder effect induced by postglacial colonization in the latter areas. Furthermore, these analyses identified the populations of Lakes Desert

(northwestern) and San Luis (south) as a group genetically distinct from the population of Lake Yehuín (the southernmost lake), suggesting that the founder populations of these lakes were different. Probably, these founder populations followed rapid population growth and local adaptation to these new and oligotrophic lakes. Therefore, as suggested by the monopolization hypothesis, the observed genetic differentiation between populations may also be due to biological barriers. Individuals may disperse well between lakes, but be unable to colonize because they cannot outcompete residents.

Conclusions

This study supported the hypothesis that freshwater microalgae have genetically structured populations. To our knowledge, this is the first study in testing this hypothesis in a lake-dwelling microalga of South America. Genetic divergence between all lake populations was moderate to high, even between geographically close populations. These populations, in turn, composed three genetically and spatially distinct groups. Marine Bathycoccaceae populations, in contrast, are known to be less influenced by distance and more by local parameters such as light intensity. Our analyses pointed out isolation by distance and isolation by environment as important drivers on the genetic structuring of microbial freshwater populations, though biological mechanisms as proposed by the monopolization hypothesis can not be ruled out.

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Table 1 Molecular diversity for the ITS region at each sampled lake in the Argentinean Patagonia. N = sample size; h = number of haplotypes; S = number of segregating sites; Hd = haplotype diversity; π = nucleotide diversity. Lakes are sorted from north to south.

Lake	Latitude (°S)	Longitude (°W)	N	h	S	Hd ($\pm$ SD)	π
Musters	45°33'00"	69°08'24"	36	4	5	0.610 $\pm$ 0.05	0.00160
Posadas	47°27'00"	71°49'12"	27	3	2	0.211 $\pm$ 0.10	0.00037
Cardiel	48°59'24"	71°07'48"	31	3	3	0.411 $\pm$ 0.09	0.00195
Desierto	49°04'47"	72°53'01"	25	4	7	0.757 $\pm$ 0.04	0.00539
San Luis	53°55'19"	67°36'59"	29	3	3	0.493 $\pm$ 0.09	0.00158
Yehuin	54°21'39"	67°46'49"	20	8	10	0.879 $\pm$ 0.05	0.00527
All	–	–	168	18	20	0.787 $\pm$ 0.03	0.00364

Table 2 Estimates of F_{ST} (below diagonal) and corresponding P values (above diagonal) based on the ITS variation between populations of the freshwater Bathycoccaceae over the Argentinean Patagonia. All F_{ST} values were significant after sequential Bonferroni correction for multiple testing ($\alpha = 0.05$). The diagonal values are zero. Lakes are sorted from north to south.

	Musters	Posadas	Cardiel	Desierto	San Luis	Yehuin
Musters	–	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Posadas	0.285	–	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cardiel	0.154	0.191	–	< 0.0001	< 0.0001	< 0.0001
Desierto	0.264	0.376	0.173	–	< 0.0001	< 0.0001
San Luis	0.615	0.771	0.465	0.240	–	< 0.0001
Yehuin	0.434	0.452	0.281	0.256	0.397	–

Table 3 Genetic divergence between populations explained by geographic and environmental distances. All values were significant after Bonferroni correction for multiple testing ($\alpha = 0.05$).

Explanatory distance matrix	<i>Response distance matrix</i>	
	R^2	P
Geographic + environmental distance	0.410	> 0.0001
Geographic distance	0.385	> 0.0001
Environmental distance	0.177	> 0.0001

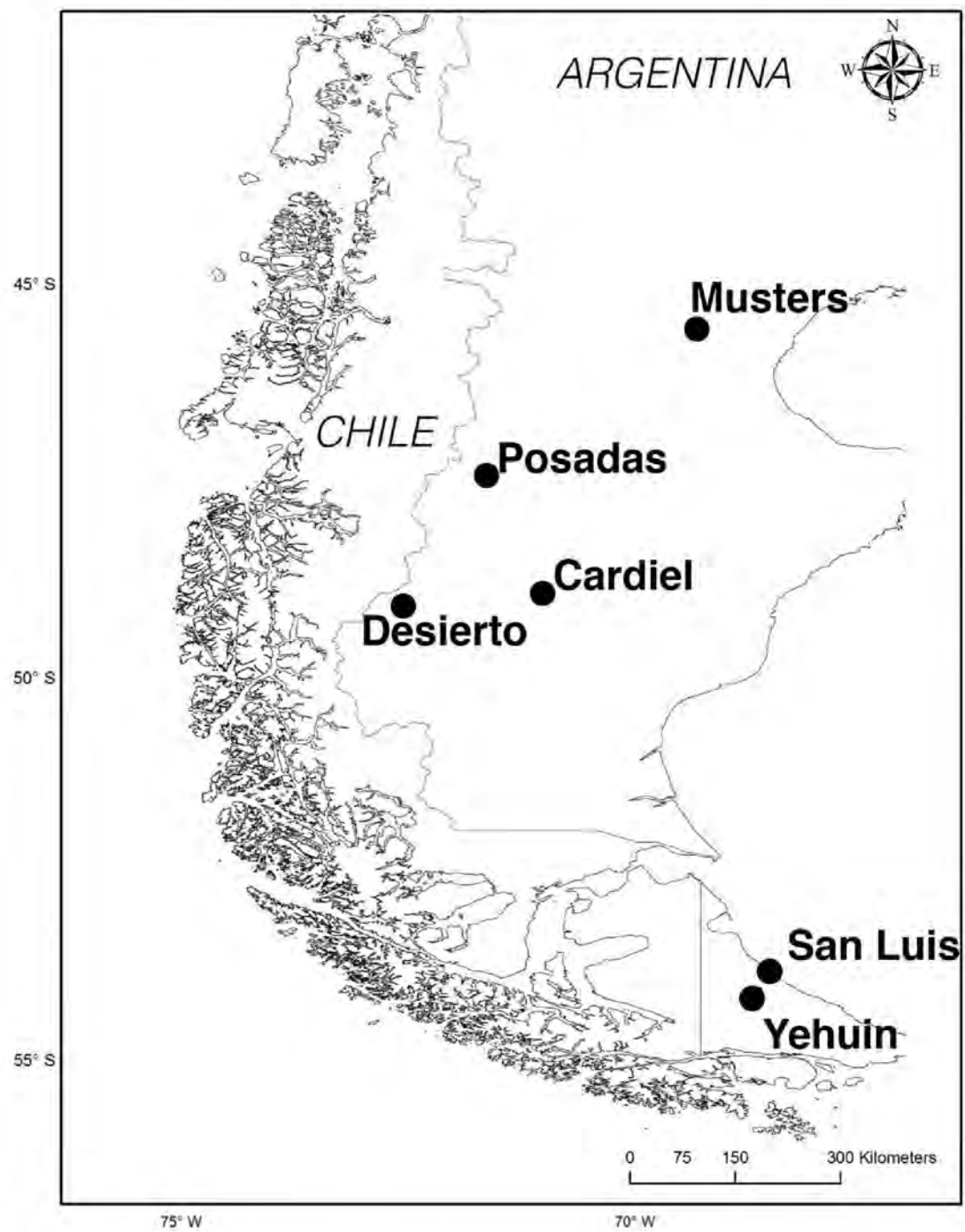


Figure 1 Map of the study site showing the geographical distribution of the six lakes studied in the Argentinean Patagonia, South America.

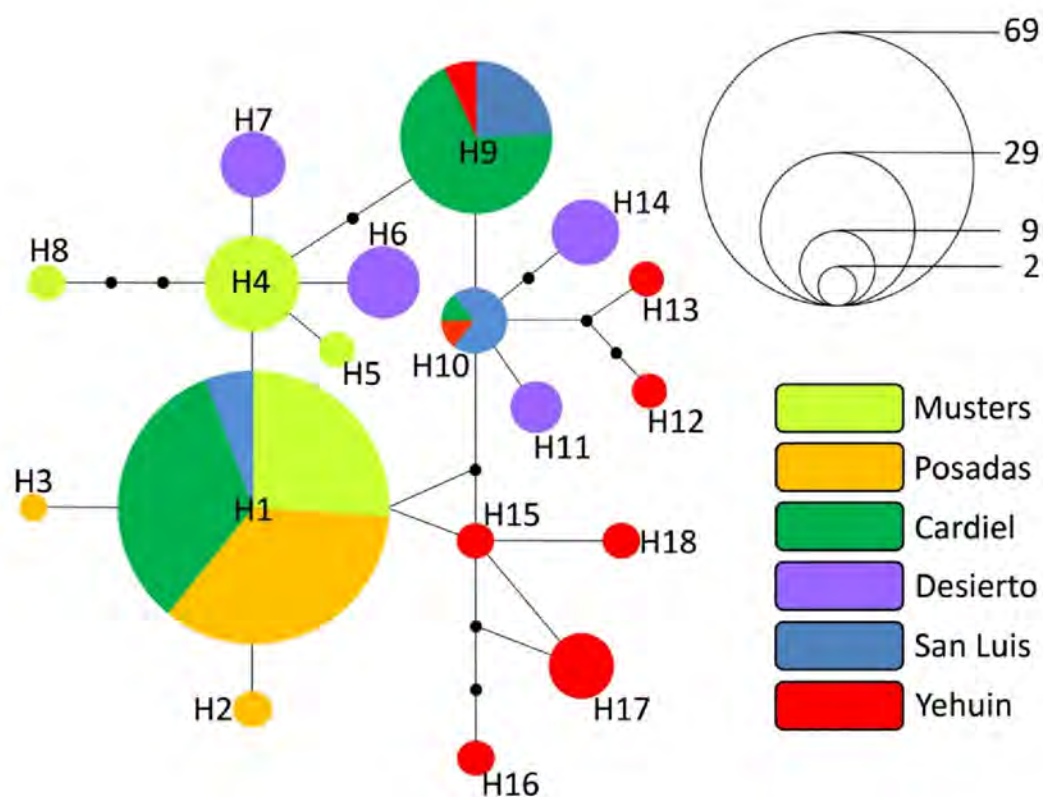


Figure 2 Haplotype network of the Argentinean freshwater Bathycoccaceae based on internal transcribed spacer operon sequences. Each haplotype has been identified with a letter-number combination and resized according to its frequency. Black dots on connecting lines indicate the number of mutational steps between haplotypes. The colours making up each haplotype represent the lacustrine origin of the sequences that compose it.

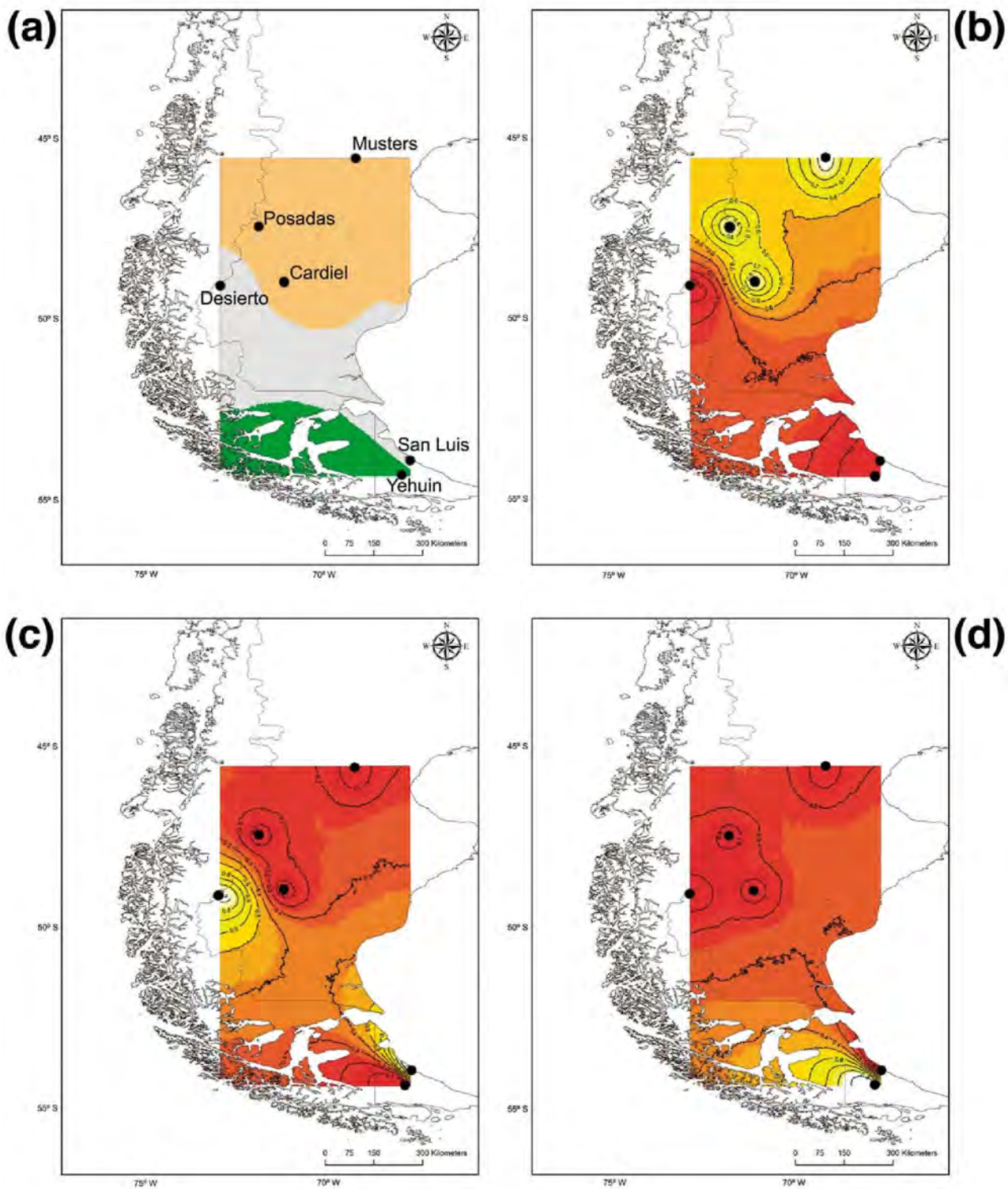


Figure 3 Spatial Bayesian clustering in the program Geneland. Geneland output has been cropped, re-scaled and superimposed on the map of the Argentinean Patagonia. Black dots depict the studied lakes. Lighter colours indicate higher probabilities of population membership. Contour lines indicate the spatial position of genetic discontinuities between populations. (a) Spatial clustering suggests three distinct clusters across the Argentinean Patagonia. Map of posterior probabilities of population membership and spatial location of genetic discontinuities for populations of the freshwater Bathycoccaceae belonging to (b) the north-central cluster; (c) the south-central cluster; and (d) the southernmost cluster.

Appendix to “A lake-dwelling microalga (Bathycoccaceae) exhibits population genetic structure among lacustrine populations due to geographic and ecological isolation”

SUPPLEMENTARY MATERIAL

A lake-dwelling microalga (Bathycoccaceae) exhibits population genetic structure among lacustrine populations due to geographic and ecological isolation

Leonardo D. Fernández^{1,2}, Cristián E. Hernández¹, M. Romina Schiaffino³, Irina Izaguirre⁴
and Enrique Lara²

Supplementary Table S1 Environmental parameters measured at each patagonian lake

	<i>Lake</i>					
	<i>Musters</i>	<i>Posadas</i>	<i>Cardiel</i>	<i>Desierto</i>	<i>San Luis</i>	<i>Yehuín</i>
Latitude (°S)	45.55	47.45	48.99	49.08	53.92	54.36
Longitude (°W)	69.14	71.81	71.13	72.89	67.60	67.78
Altitude (m a.s.l.)	277	160	280	506	10	169
Temperature (°C)	12.60	10.00	11.00	8.20	10.70	8.00
pH	7.84	7.84	9.30	6.90	8.10	8.00
Conductivity ($\mu\text{S cm}^{-1}$)	310	210	4360	20	313	197
OD (ppm)	9.3	9.8	10.3	11.9	10.7	11.5
SRP (ppm)	0.38	0.07	1.39	0.15	0.17	0.04
DIN (ppm)	0.23	0.05	0.10	0.14	0.08	0.14
Kd (m^{-1})	1.153	0.089	0.232	0.123	2.060	0.405
DOC (ppm)	10.8	6.1	11.8	5.1	17.8	9.3
Chl a ($\mu\text{g l}^{-1}$)	2.62	0.38	0.76	0.20	0.68	0.10

Chapter 6: Discussion and conclusions

Discussion and conclusions

Gaps in knowledge

Over the last decade, several studies have shown that multicellular (plants and animals) and unicellular (protists) eukaryotes may have similar broad-scale patterns of diversity (Bates et al., 2013; Heger et al., 2015; Fernández et al., 2016; Fournier et al., 2016; Lara et al., 2016; Schiaffino et al., 2016; Wang et al., 2016) and population genetic structure (Ryner and Armbrust, 2004; Nagai et al., 2007; Godhe and Harnström, 2010; Harnström et al., 2011; Sjöqvist et al., 2015). However, despite this, it is still discussed whether this finding might be generalized to all protists. Most studies have only investigated these patterns in ciliates, diatoms and dinoflagellates of the Holartic region (Fenchel and Finlay, 2003), while other protist groups and regions of the planet remain comparatively much less studied (Haman and Kohl, 1994; Fernández et al., 2015; Sigala et al., 2016).

Moreover, it is still debated whether these patterns are produced and maintained by similar causes in multicellular and unicellular eukaryotes (Foissner and Hawksworth, 2009; Fontaneto, 2011). Currently, broad-scale diversity patterns of plants and animals are explained in terms of ecological and long-term processes (Mittelbach et al., 2007). By contrast, broad-scale diversity patterns of protists are often only explained in terms of ecological processes. In particular, it has been suggested that in protists, these diversity patterns are driven by environmental factors related to the availability of water and/or ambient energy (e.g. Bates et al., 2013; Heger et al., 2015; Lara et al., 2016; Wang et al., 2016). While this finding represents a major contribution towards a better understanding of the causes that underlie broad-scale diversity patterns in protists, it does not allow determining the role played by long-term processes. Finally, few studies have explicitly investigated the patterns and causes of population genetic structure in protists (Nagai et al., 2007; Godhe and Harnström, 2010; Harnström et al., 2011; Sjöqvist et al., 2015).

Contributions of this thesis

Our limited knowledge on the patterns and causes that originate and maintain broad-scale patterns of diversity and population genetic structure in protists has limited the construction of unifying ecological generalizations between multicellular and unicellular organisms

(Martiny et al., 2006). In this context, this thesis represented a significant contribution because it provided valuable information that helped to meet this need.

Chapter 1 revealed that soil testate amoeba diversity is not randomly distributed in the Chilean territory. In continental Chile, only 11% of the species were widely distributed, while the remaining 89% exhibited more restricted geographical distributions. The latter species were mainly restricted to the central zone of continental Chile, which, is characterised by regular precipitation and mild temperatures. The central zone of continental Chile is also part of a major biodiversity hotspot, harbouring a high number of endemic and endangered multicellular species. Consistently with this fact, central Chile exhibited an important number of endemic testate amoeba species (ca. 10%). Given that the occurrence of endemisms are produced and maintained by the joint influence of ecological and long-term processes (Brown and Lomolino, 1998), this chapter suggested that soil testate amoeba diversity and distribution might also be driven by these two mechanisms.

Chapters 2 and 3 demonstrated that the patterns and causes of broad-scale diversity in protists of the northern and southern hemispheres are similar to those observed in multicellular organisms. Chapter 2 found that in southwestern South America (Chile) soil testate amoebae have a unimodal diversity gradient with a peak of richness at mid-latitudes, coupled with abrupt declines in richness towards lower and higher latitudes. This unusual latitudinal gradient in diversity is also exhibited by several plants and animals of this region (e.g. Villagrán and Hinojosa, 1997; Hernández et al., 2005; Löwenberg-Neto et al., 2008; Samaniego and Marquet, 2009; Fuentealba et al., 2010; Bannister et al., 2012; Lee and Riveros, 2012; Palma, 2013; Segovia et al., 2013; Parra et al., 2015). Chapter 3 found that both well-studied and less-studied groups of soil protists (Bacillariophyta, Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida) exhibit a decrease in richness with elevation in the Swiss lowlands and Jura Mountains. This elevational diversity pattern has been repeatedly observed in several groups of multicellular organisms around the world (reviewed in Rahbek, 1995, 2005; McCain, 2007, 2009, 2010). In line with other previous studies (Bates et al., 2013; Heger et al., 2015; Lara et al., 2016; Wang et al., 2016), both chapters also found that protist diversity peaked in sites with the warmest and wettest conditions. Complementary analyses revealed that this common trend might stem from an ancestral adaptation of soil protists to tropical-like conditions. Put in another way, these analyses suggested that soil protists will always be

more diverse in humid and warm sites just because their evolutionary history will impose them ecophysiological constraints that will prevent most of them from colonizing cold and hot sites. This phylogenetic conserved response to water-energy availability has proved useful in explaining the occurrence of broad-scale diversity patterns in plants (Bryant et al., 2008; Qian et al., 2013; Anacker et al., 2014; Ma et al., 2014) and animals (Wiens and Donoghue, 2004; Hawkins et al., 2006; Wiens et al., 2010; Hortal et al., 2011). Therefore, this finding provided yet another proof that the diversity of unicellular and multicellular eukaryotes is driven by similar processes.

Finally, chapter 4 demonstrated that the pattern and causes of population genetic structure in protists are also similar to those observed in multicellular organisms. In detail, a lake-dwelling eukaryotic microalga showed moderate to high levels of differentiation between lake populations distributed along the Argentinean Patagonia. Further, a spatially explicit Bayesian clustering model grouped lake populations in three distinct genetic unities, confirming that there was population genetic structure. A multiple regression analysis on matrices of genetic, geographic and environmental distances revealed that population differentiation might be the result of isolation by distance and isolation by environment. Moreover, there was evidence supporting the idea that differentiation between lake populations might also stem from a post-glacial (historical) founder effect in southern Patagonia. These results confirmed that just like multicellular organisms, protists do not have unlimited dispersal and gene flow. Also confirmed that landscape features (including historical barriers to dispersal represented by the ice sheet of the Last Glacial Maximum), might play an important role on their population genetic structure.

In summary, this thesis confirmed that protists also have broad-scale patterns of diversity and population genetic structure. It also demonstrated that this finding might not only be valid for protists of the Holarctic region, but also for those occurring in sub-sampled regions of the planet, such as southern South America. It also showed that these patterns are valid for both well- and less-studied groups of protists. Finally, this thesis confirmed that in protists these patterns are produced and maintained by a combination of ecological and long-term processes. Therefore, this thesis provided strong support for the hypothesis that, at broad spatial scales, the patterns and underlying causes of diversity and population genetic structure of protists are similar to those observed for multicellular organisms. Moreover, the model organisms used in this thesis are good representatives of the enormous diversity of trophic, morphological, locomotive, physiological and probably

reproductive strategies exhibited by protists. Therefore, the acceptance of this hypothesis could be valid for a large number of eukaryotic microorganisms.

Future work

This PhD thesis contributed to determine the patterns and causes that originate and maintain the patterns of diversity and population genetic structure in protists. Future studies should re-assess the findings of the present research in other microbial groups and over broader spatial scales. For instance, it would be interesting to re-assess the patterns and causes of latitudinal gradients in protist diversity from the tropic to the poles; or the patterns and causes of altitudinal gradients in protist diversity from the sea level to the top of mountains subjected to different climatic contexts. It would also be enlightening to re-assess the patterns and causes of population genetic structure in areas characterised by strong climatic gradients and habitats with different degrees of interconnectivity. Together, these analyses could help to better understand the relative role played by ecological and long-term processes in shaping microbial diversity at broad-spatial scales.

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Appendices: Side projects in which I was involved during my PhD

Appendix A. Microbial eukaryote communities exhibit robust biogeographical patterns along a gradient of Patagonian and Antarctic lakes

Microbial eukaryote communities exhibit robust biogeographical patterns along a gradient of Patagonian and Antarctic lakes

M. Romina Schiaffino,^{1*} Enrique Lara,²
Leonardo D. Fernández,^{2,3,4} Vanessa Balagué,⁵
David Singer,² Christophe C. W. Seppey,²
Ramon Massana⁵ and Irina Izaguirre⁶

¹Centro de Investigaciones y transferencia del Noroeste de la Provincia de Buenos Aires, CONICET, UNNOBA, Junín, 6000, Argentina.

²Laboratory of Soil Biodiversity, University of Neuchâtel, Rue Emile Argand 11, Neuchâtel, CH-2000, Switzerland.

³Laboratorio de Ecología Evolutiva y Filoinformática, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario s/n, Concepción, Chile.

⁴Facultad de Medicina Veterinaria y Agronomía, Universidad de Las Américas, Sede Concepción, Chacabuco 539, Concepción, Chile.

⁵Institut de Ciències del Mar, CSIC, Passeig Marítim de la Barceloneta 37-49, Barcelona, Catalonia, 08003, Spain.

⁶Departamento de Ecología, Genética y Evolución, IEGEBA (CONICET-UBA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Buenos Aires, C1428EHA, Argentina.

Summary

Microbial eukaryotes play important roles in aquatic ecosystem functioning. Unravelling their distribution patterns and biogeography provides important baseline information to infer the underlying mechanisms that regulate the biodiversity and complexity of ecosystems. We studied the distribution patterns and factors driving diversity gradients in microeukaryote communities (total, abundant, uncommon and rare community composition) along a latitudinal gradient

of lakes distributed from Argentinean Patagonia to Maritime Antarctica using both denaturing gradient gel electrophoresis (DGGE) and high-throughput sequencing (Illumina HiSeq). DGGE and abundant Illumina operational taxonomic units (OTUs) showed both decreasing richness with latitude and significant differences between Patagonian and Antarctic lakes communities. In contrast, total richness did not change significantly across the latitudinal gradient, although evenness and diversity indices were significantly higher in Patagonian lakes. Beta-diversity was characterized by a high species turnover, influenced by both environmental and geographical descriptors, although this pattern faded in the rare community. Our results suggest the co-existence of a 'core biosphere' containing reduced number of abundant/dominant OTUs on which classical ecological rules apply, together with a much larger seedbank of rare OTUs driven by stochastic and reduced dispersal processes. These findings shed new light on the biogeographical patterns and forces structuring inland microeukaryote composition across broad spatial scales.

Introduction

Phototrophic and heterotrophic small eukaryotes of picoplanktonic, nanoplanktonic and microplanktonic size (i.e. microbial eukaryotes with a size ranging between 0.2 and <20 µm) are abundant in the upper photic zone of all lakes and oceans (Li *et al.*, 1994; Caron *et al.*, 1999; Foissner, 1999) and play important roles in the biogeochemical cycles (e.g. Pomeroy, 1974; Azam *et al.*, 1983; Stockner and Antia, 1986; Massana, 2011). The introduction of molecular techniques into microbial ecology has greatly increased our knowledge on microbial eukaryotes diversity (e.g. Epstein and López-García, 2008). Fingerprinting techniques, such as denaturing gradient gel electrophoresis (DGGE), allow treating a relatively higher number of samples (van Hannen *et al.*, 1998), but provide limited information on the taxonomical composition of microbial communities and retrieve only the abundant or dominant

Received 8 June, 2016; accepted 30 September, 2016. *For correspondence. E-mail: rschiaffino@conicet.gov.ar; Tel. (+54 236) 4638745.

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taxa, the so called 'core species' (Magurran and Henderson, 2003). In the last years, the introduction of novel high-throughput sequencing technologies permitted reaching saturation of microbial diversity, including the rarest taxa (the so-called 'rare biosphere', e.g. Sogin *et al.*, 2006; Dawson and Hagen, 2009; Monchy *et al.*, 2011; Lepère *et al.*, 2013; Taib *et al.*, 2013; Logares *et al.*, 2014).

Diversity is considered a key property of ecosystems, necessary to understand the structure, functioning and dynamics of communities (e.g. MacArthur and Wilson, 1967; Magurran, 2004). It comprises two basic components, taxa richness (number of taxa in a community) and evenness (a measure of how similar taxa are in their abundances) (Magurran, 2004). Weak relationships between these two components in many aquatic datasets reflect independent components of diversity, suggesting that richness alone may be an incomplete surrogate for biodiversity (Soininen *et al.*, 2012). Thus, richness and evenness should be treated separately (e.g. Hurlbert, 1971; Magurran, 1988; Legendre and Legendre, 1998) because both provide meaningful insights into community function (McNaughton, 1977) and may respond independently to different ecological processes (Ma, 2005). Beta-diversity (Whittaker, 1960) is usually applied in a broad sense to any measure of variation in taxa composition between sites (Anderson *et al.*, 2011). As suggested by Baselga (2010; 2012), it can be partitioned into two components: turnover (the replacement of some taxa by others from site to site) and nestedness (the pattern characterized by the poorest site being a strict subset of the richest site, without replacement of taxa).

Ecological communities are also characterized by a few species exceptionally abundant, while the majority often remains remarkably rare (Fisher *et al.*, 1943; Preston, 1948; Magurran, 2004). Therefore, communities can be separated into two components: 'core species', which are persistent and abundant, and 'rare or occasional species', which occur infrequently in the record and are typically low in abundance (Magurran and Henderson, 2003). This pattern is repeated across taxonomic groups (Magurran, 2004), including planktonic microorganisms (e.g. Pedrós-Alíó, 2006; Mangot *et al.*, 2013). These two components are not independent from each other, and rare taxa can be promoted from the 'rare biosphere' to the 'core biosphere' if conditions alter sufficiently and become adequate for the growth of a particular taxon. Likewise, a member of the 'core biosphere' can be grazed down or reduced by viral lysis below the threshold level to become a member of the 'rare biosphere' without disappearing from the ecosystem (Pedrós-Alíó, 2006).

Recent studies have aimed to characterize the patterns of this 'rare biosphere' in ecosystems (Lepère *et al.*, 2013; Logares *et al.*, 2014). According to the 'seed bank theory' and as mentioned before, the 'rare biosphere' is supposed

to play the role of a large inactive seed bank, dormant most of the time (e.g. as cysts or spores), and awaiting adequate conditions to develop (Lennon and Jones, 2011; Gibbons *et al.*, 2013). Previous studies in freshwater seasonal variation (Lara *et al.*, 2011; Simon *et al.*, 2015) suggested that members of the eukaryotic community may enter some way of dormancy and constitute a 'seed bank' that participates to plankton community resilience over time. Furthermore, Simon *et al.* (2016) recently observed that the capacity to enter dormancy via the generation of resting forms (e.g., cysts, spores or other resistant metabolic stages) is the major explanation for the resilience of microbial communities observed in shallow freshwater ecosystems undergoing frequent droughts. Since a dormant microbial cell can survive for a long time in sub-optimal environments and can be transported passively over large distances in the meantime, some researchers argued that microbial species would have a cosmopolitan distribution (Finlay, 2002). The implications of these assumptions are twofold. First, it would mean that the large spatial diversity patterns observed in the 'rare biosphere' are independent of the forces that drive current diversity patterns in other life-forms (e.g. plants and animals), such as environmental factors and isolation by distance. Second, it would mean that beta-diversity patterns (i.e. variation in OTU composition among sites) will tend to be null. This globally more or less homogeneous rare and inactive biosphere would then bring only redundant information between different high-throughput sequencing surveys, and early molecular techniques such as DGGE would suffice to describe the biologically relevant part of diversity. Thus, if it were true that all microbes are easily dispersed globally, the long tail of the distribution of species abundances in any ecosystem would include all of the microorganisms on Earth, and the biodiversity of any ecosystem would be identical to the biodiversity on the planet (Pedrós-Alíó, 2006). However, recent studies do not support these assumptions. It has been shown in freshwater (Debroas *et al.*, 2015) and marine (Logares *et al.*, 2014) systems that at least some of the rare microorganisms are active, and that microbial eukaryotes from high-mountain lakes are not globally distributed (e.g. Filker, *et al.*, 2016). In addition, studies focusing on marine prokaryotes showed that rare OTUs followed similar distribution patterns to those of the most abundant members of the community and of the entire community (Galand *et al.*, 2009; Logares *et al.*, 2013). Similar patterns have been also observed for marine and freshwater microbial eukaryotes (Lepère *et al.*, 2013; Logares *et al.*, 2014).

The profound influence of microorganisms on human life and global biogeochemical cycles underlines the value of studying the biogeography of microorganisms (Lynch and Neufeld, 2015). The most common pattern in biogeographical studies is the decrease of richness with

Table 1. Comparisons between cell abundances and diversity properties of microbial eukaryotes from Patagonian and Antarctic water bodies.

			Patagonian lakes	Antarctic lakes
Epifluorescence	Autotrophic abundance	$n = 40$	$7.7 \times 10^{4***}$	$5.4 \times 10^{3***}$
	Heterotrophic abundance		$1.1 \times 10^{4**}$	$2.2 \times 10^{3**}$
DGGE	Dominant OTU richness	$n = 40$	21*	18*
	Dominant OTU richness	$n = 14$	20	18
Illumina HiSeq	Total OTU richness	$n = 14$	347	313
	Chao 1		539	493
	Abundant OTUs $\geq 1\%$ richness		19**	10**
	Uncommon OTUs >0.1 to $<1\%$ richness		52	38
	Rare OTUs $\leq 0.1\%$ richness		276	265
	Simpson Index (D)		13.4*	6.7*
	Shannon Index (H')		3.4*	2.6*
	Evenness (E_D)		0.041	0.022
	Evenness (E_H)		0.584*	0.445*

Average per lake, $n = 40$ (28 Patagonian and 12 Antarctic water bodies) and $n = 14$ (8 Patagonian and 6 Antarctic water bodies).

Student's *t*-test * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

increasing latitude, although this pattern may depend on the spatial scale and the level of taxonomic resolution (e.g. Willig *et al.*, 2003; Hillebrand, 2004). However, there are no clear geographical patterns in relation to evenness and contrasting patterns have been reported (e.g. Willig *et al.*, 2003). South American and Antarctic lakes are propitious systems to study the diversity distribution patterns in microorganisms. Indeed, these freshwater systems can be found along a significant latitudinal gradient that exhibits relatively benign (low latitudes) to extremely harsh (higher latitudes) environmental conditions, as well as important barriers to dispersal (e.g. the Magellan Strait and the Drake Passage), that allow not only assessing their diversity patterns but also the importance of environmental and geographical factors in determining these patterns. Previous studies on microbial diversity patterns across Patagonian and Antarctic lakes have found a decreasing latitudinal diversity gradient for diatoms (Maidana *et al.*, 2005), Chlorococcales (Tell *et al.*, 2011), phytoplankton (Izaguirre *et al.*, 2016), and bacterioplankton (Schiaffino *et al.*, 2011).

Here, we have used both traditional fingerprinting (DGGE) and high-throughput sequencing technology (Illumina HiSeq) methods to study the patterns and drivers of latitudinal diversity gradients in total, abundant, uncommon and rare microbial eukaryote community along a gradient of freshwater environments (Fig. 1) stretching from Argentinean Patagonia (45° S) to Maritime Antarctica (63° S). We used DGGE, which focused on the dominant taxa, on the complete dataset (40 lakes), while the Illumina HiSeq approach that allowed reaching complete taxonomical reports was applied on a subset of samples (14 lakes). The use of both approaches provided a unique opportunity to compare their performance and conclusions. For both datasets, we hypothesised that OTU richness and diversity follow a decreasing

pattern with increasing latitude, environmental harshness and geographical distance, just as it has been repeatedly shown for several other taxa (Brown and Lomolino, 1998; Gaston, 2000). In addition, given that environments from lower latitudes are less extreme and more productive than those from higher latitudes (Hawkins *et al.*, 2003; Currie *et al.*, 2004; Chase and Ryberg, 2004), we proposed that there are a higher proportion of dominant OTUs and evenness in lower latitudes than in higher latitudes. Furthermore, important changes in community compositions are assumed between Patagonian and Antarctic lakes due to environmental and geographical factors, which will be reflected by beta-diversity. Alternatively, we postulated that beta-diversity patterns of the rare biosphere are independent of the environmental and/or geographical distances, giving support to the 'seed bank theory'.

Results

Total abundances

In most water bodies, the abundance of autotrophic eukaryotes was greater than the abundance of heterotrophic ones (both $\leq 5 \mu\text{m}$ in diameter, Table 1), and both showed the same behaviour with respect to the trophic and latitudinal gradients (Table 2). Patagonian water bodies presented significantly higher autotrophic and heterotrophic eukaryotes abundances than Antarctic ones (Table 1). In addition, the abundance of autotrophic and heterotrophic eukaryotes decreased significantly towards higher latitudes and accordingly increased significantly with temperature and higher chl *a* (Table 2).

DGGE analysis

The DGGE gels performed for 40 lakes yielded a total of 819 bands located in 90 different positions (total dominant

Table 2. Spearman Rho correlations between microbial eukaryote properties (abundance, richness and diversity) and some environmental variables from all the studied water bodies.

		Lake area	Latitude	Temperature	Chlorophyll a
Epifluorescence (<i>n</i> = 40)	Autotrophic abundance	0.16	-0.61**	0.67**	0.42*
	Heterotrophic abundance	-0.31	-0.46*	0.56**	0.47*
DGGE	Dominant OTU richness (<i>n</i> = 40)	0.11	-0.39	0.14	0.34
	Dominant OTU richness (<i>n</i> = 14)	0.08	-0.32	0.15	0.59
Illumina HiSeq (<i>n</i> = 14)	Total OTU richness	-0.04	-0.01	0.10	-0.10
	Chao 1	-0.02	0.01	0.10	-0.29
	Abundant OTUs $\geq 1\%$ richness	0.74*	-0.74*	0.58	-0.22
	Uncommon OTUs >0.1 to $<1\%$ richness	0.08	-0.18	0.22	0.19
	Rare OTUs $\leq 0.1\%$ richness	-0.15	0.13	0.07	-0.20
	Simpson Index	0.48	-0.39	0.21	0.01
	Shannon Index	0.41	-0.39	0.30	0.05
	Evenness (E_D)	0.47	-0.29	0.10	-0.06
	Evenness (E_H)	0.54	-0.53	0.40	0.02

n = 40 for DGGE and epifluorescence microscopy counts, and *n* = 14 for Illumina HiSeq reads and DGGE data.

Bold values represent $P < 0.05$, * $P < 0.01$, ** $P < 0.0001$.

richness): 24 positions (27%) were exclusive in Patagonian lakes and 3 (3%) in Antarctic lakes, whereas 63 positions (70%) were shared between Patagonian and Antarctic water bodies. We successfully sequenced 26 different OTUs (representing 40% of the total intensity of the gels). Bands with the same position were sequenced from different lakes to confirm that they corresponded to the same sequence: e.g. bands 3 (a, b), 7 (a, b) and 17 (a, b) (Fig. 2). We found members of five taxonomic groups: 10 (38%) Stramenopiles (8 Chrysophyceae-Synurophyceae, 1 Bicosoecida, 1 Dictyochophyceae), 10 (38%) Alveolata (1 Alveolata, 8 Ciliophora and 1 Apicomplexa), 3 (12%) Archaeplastida (Chlorophyta), 2 (8%) Fungi and 1 (4%) Telonemida (Supporting Information Table S1). Some sequences showed their closest match and phylogenetic affiliation (between 98 and 100% of similarity) with well-described species of small phytoplankton (generally smaller than 5 μm) *Vitreochlamys incisa* (band 15), *Pyramimonas australis* str. (band 16), *Ostreococcus tauri* (band 26). Other sequences matched with OTUs belonging to taxonomic groups represented by larger cell sizes (e.g. Alveolata), possibly due to the inefficient sequential filtration (Supporting Information Table S1).

Illumina HiSeq analysis

From a total of 5 552 925 paired-end sequences, 3 670 418 passed the quality check. The total richness across all studied lakes was 1 505 different OTUs and ranged from 147 to 475 OTUs in each individual water body (332 ± 99 OTUs on average). Rarefaction curves performed for every water body showed that OTUs richness tended to reach a plateau, indicating that the sampling depth and sequencing coverage were good (Fig. 3). In general, Stramenopiles and Alveolata dominated diversity in the entire study (Fig.

4). Taxonomic composition showed clear differences between Patagonian and Antarctic water bodies: Cryptophyta were abundant in Patagonia but absent from Antarctic lakes, whereas Chrysophyta dominated in Antarctic lakes (Fig. 4). Ciliophora showed higher relative abundances and were more frequent in Patagonian than in Antarctic lakes, except in the Antarctic Pingüi Pond (Fig.



Fig. 1. Map showing the 40 locations of the studied water bodies. All sites were studied with DGGE, while those that also were studied with Illumina HiSeq (14 lakes) are shown with white circles.

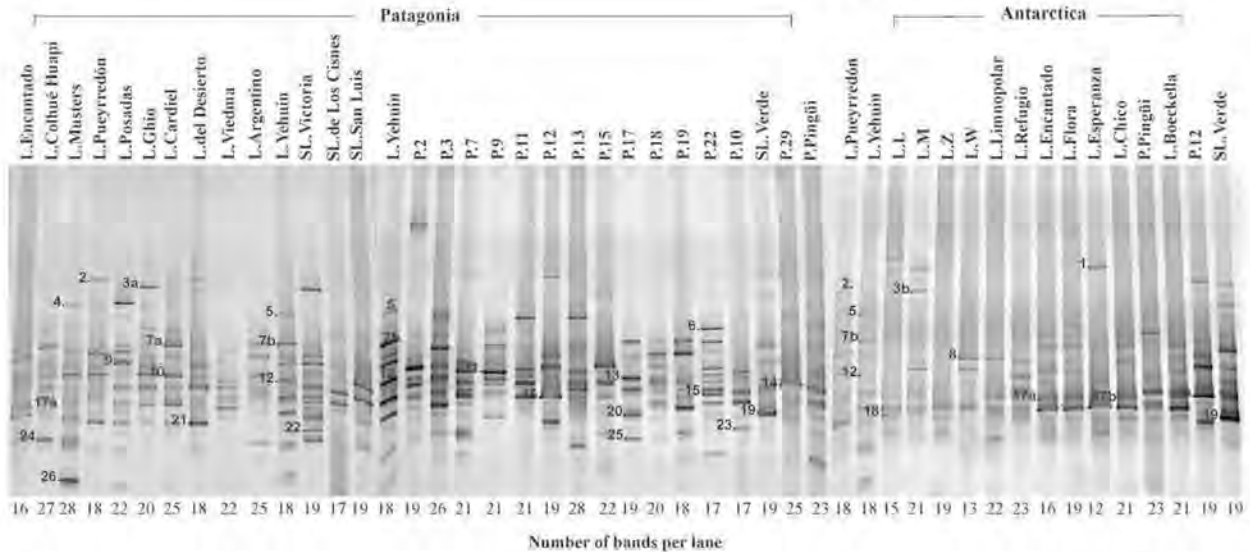


Fig. 2. Image of the DGGE gels run for 40 water bodies. Numbers in the gels indicate excised and sequenced bands. L.: lake, SL.: shallow lake, P.: pond.

4). This system has a large contribution of organic matter from penguins and represents an outlier in our dataset.

Globally, the 'uncommon and rare biosphere' presented a high percentage (92%) of exclusively uncommon or rare OTUs, whereas the 'core biosphere' showed a low percentage (4%) of exclusively dominant/abundant OTUs. Therefore, from the total 125 'core OTUs' found in 14 lakes, 120 (96%) were dominant in some lakes but also rare in other lakes, whereas only 5 (4%) were not found as rare in other lakes. In addition, 67% of these 125 OTUs were dominant just in one lake and 33% were dominant in two or more lakes (up to 6) lakes, and none of these OTUs was dominant in all studied lakes. On the other hand, from the total 1500 OTUs found in the 'uncommon and rare biosphere' (OTUs $\leq 1\%$), 1380 OTUs (92%) were always

uncommon and/or rare (exclusive) while 120 of these OTUs (8%) were also found in the 'core biosphere' in other lakes. In all samples, we found a small number (around 8%) of abundant OTUs ($\geq 1\%$), while most OTUs (around 92%) were rare (OTUs $\leq 0.1\%$). Patagonian lakes showed significantly higher numbers of dominant OTUs ($\geq 1\%$) than Antarctic ones, whereas Antarctic lakes showed significantly higher numbers of rare OTUs ($\leq 0.1\%$) than Patagonian lakes (Fig. 5). Comparisons among each abundance category (total Illumina matrix, OTUs $\geq 1\%$, OTUs $> 0.1\%$ to $< 1\%$ and OTUs $\leq 0.1\%$) for all Patagonian and Antarctic water bodies are shown in Table 3. Patagonian lakes showed between 43 and 73% of exclusive OTUs, while Antarctic lakes showed between 24 and 33%. Patagonian and Antarctic lakes shared between 2 and

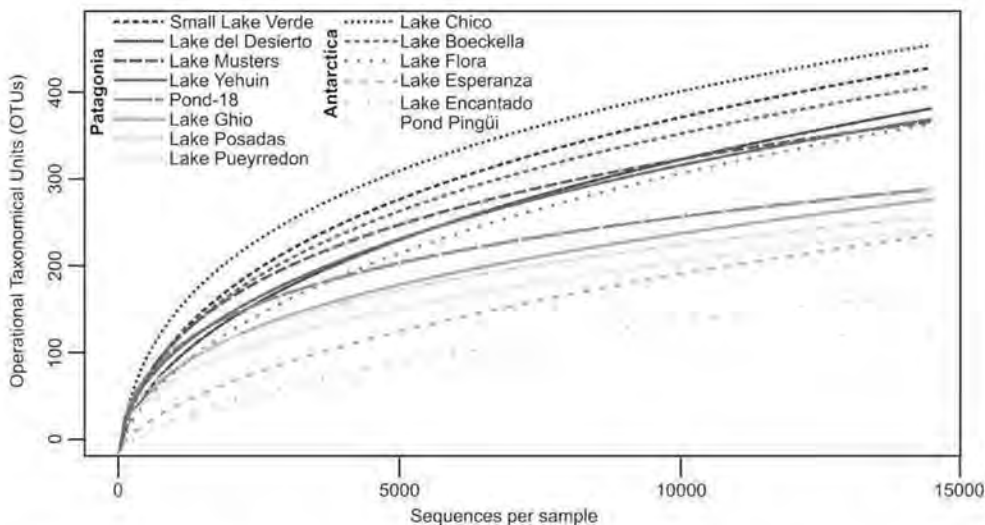


Fig. 3. Rarefaction curves for each water body using Illumina HiSeq dataset.

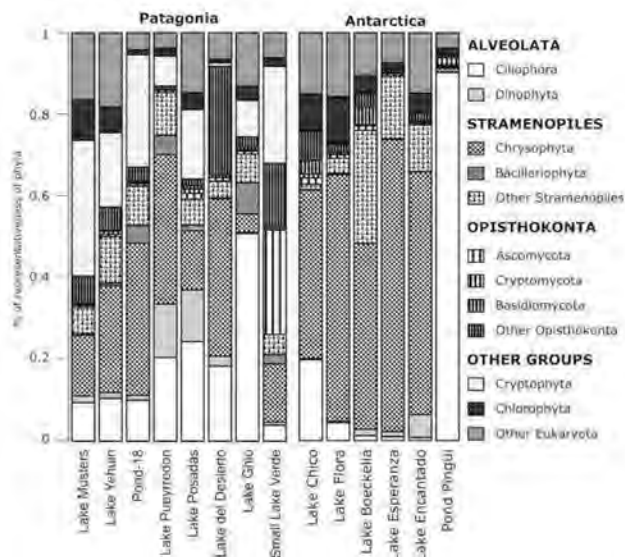


Fig. 4. Relative composition of phyla from the 14 water bodies studied with Illumina HiSeq.

32% OTUs. For instance, the abundant OTUs community composition ($\geq 1\%$) showed a total of 125 OTUs and only 2 OTUs belonging to Chrysophyceae were equally dominant in both Patagonian and Antarctic water bodies. All other abundant OTUs (123 OTUs) could dominate either in Antarctic (32) or in Patagonian (91) lakes, but never in both. Contrarily, the rare community composition ($\leq 0.1\%$) showed a total of 1438 OTUs. From these OTUs, 461 were present in both Patagonian and Antarctic lakes, whereas 977 OTUs could only be present in Antarctic (359) or in Patagonian (618) lakes. This abundance category showed that only 3 OTUs (related to Fungi) were present in all water bodies.

To compare the responses of the individual diversity components (richness and evenness) we studied the relationship between them. No correlations between richness

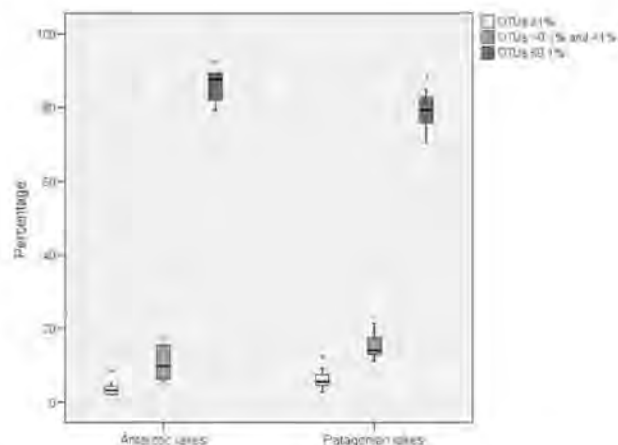


Fig. 5. Average percentage per lake of the abundant, uncommon and rare OTUs in Patagonian and Antarctic lakes. Illumina community matrix was studied considering the abundant OTUs $\geq 1\%$ of the total number of sequences in a given sample and the less frequent OTUs with two different criteria: uncommon OTUs $> 0.1\%$ to $< 1\%$ and rare OTUs $\leq 0.1\%$ of the total number of sequences in a given sample. *Student's *t*-test $P < 0.05$ for comparing each abundance category (OTUs $\geq 1\%$, OTUs $> 0.1\%$ to $< 1\%$ and OTUs $\leq 0.1\%$) between Patagonian and Antarctic lakes.

and evenness were found ($r = -0.07$ for E_D and $r = 0.39$ for E_H , both $P > 0.05$ and $n = 14$). Similar lack of relationship was found when studying the Patagonian lakes ($r = -0.41$ for E_D and $r = -0.27$ for E_H , both $P > 0.05$ and $n = 8$), or the Antarctic lakes separately ($r = 0.26$ for E_D and $r = 0.77$ for E_H , both $P > 0.05$ $n = 6$).

General patterns and comparisons

The abundant part of Illumina reads and the number of DGGE bands were significantly higher in Patagonian water bodies than in Antarctic ones (Table 1), and also decreased significantly with higher latitudes (Table 2). On the other hand, the total richness (as shown by Illumina

Table 3. Total, exclusive and shared number of sequence reads (Illumina HiSeq) for all Patagonian and Antarctic water bodies.

	Total OTUs	Shared OTUs	Exclusive OTUs	OTUs present in all water bodies	OTUs present in one water body
Total matrix					
Patagonian lakes	1 505	486	663	24	445
Antarctic lakes			356		
Abundant OTUs $\geq 1\%$					
Patagonian lakes	125	2	91	0	84
Antarctic lakes			32		
Uncommon OTUs $> 0.1\%$ to $< 1\%$					
Patagonian lakes	422	24	257	1	295
Antarctic lakes			141		
Rare OTUs $\leq 0.1\%$					
Patagonian lakes	1 438	461	618	3	506
Antarctic lakes			359		

Total OTU richness from 14 water bodies: 8 Patagonian and 6 Antarctic ones.

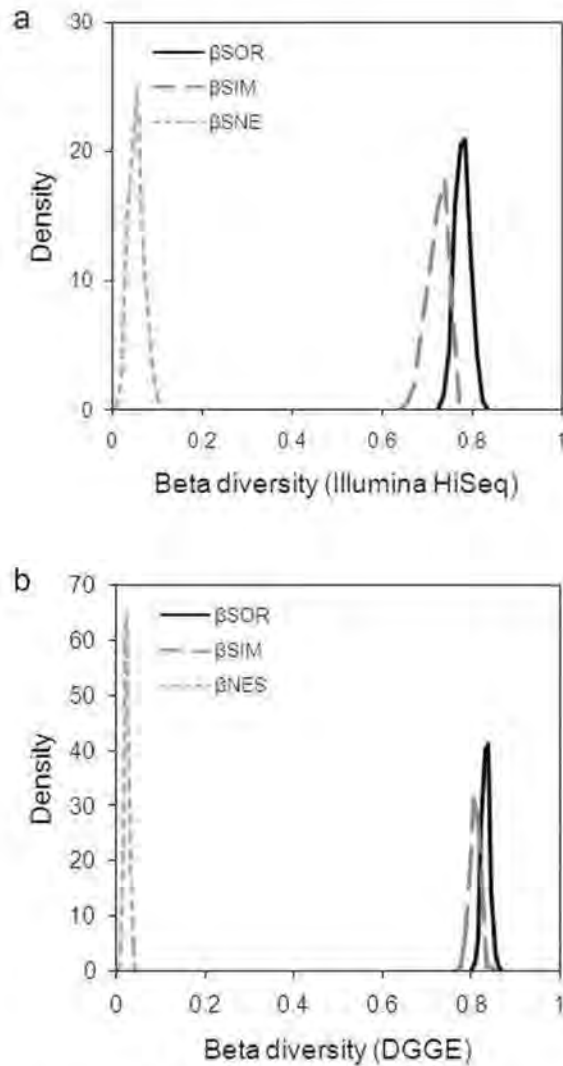


Fig. 6. Mechanisms driving variation in OTU composition in Patagonian–Antarctic lakes. The plots show the distribution of dissimilarity values due to overall beta-diversity (β_{SOR}), turnover (β_{SIM}) and nestedness (β_{NES}) based on (a) Illumina HiSeq and (b) DGGE data sets (a) Illumina HiSeq and (b) DGGE data sets, both $n = 14$ water bodies. Kernel density curves were constructed by re-sampling four lakes from each area 1000 times and computing the average β_{SOR} , β_{SIM} and β_{NES} respectively.

data) did not differ between Patagonian and Antarctic lakes (Table 1) and neither correlated with latitude (Table 2). Similar results were obtained with the richness estimator Chao 1 index (Tables 1 and 2). Both OTU diversity indices and evenness (E_H) were significantly higher in Patagonian water bodies than in Antarctic ones, whereas neither diversity indices nor evenness changed with latitude, except Pielou evenness index (E_H) that decreased with higher latitudes (Tables 1 and 2).

Microbial eukaryote communities exhibited high values of beta-diversity either based on DGGE ($\beta_{SOR} = 0.94$, $P < 0.001$) or on Illumina ($\beta_{SOR} = 0.84$, $P < 0.001$)

datasets. In both cases, variation in community composition was mainly due to OTUs turnover (DGGE $\beta_{SIM} = 0.93$ and Illumina $\beta_{SIM} = 0.86$, both $P < 0.05$) and barely due to nestedness (DGGE $\beta_{NES} = 0.01$ and Illumina $\beta_{NES} = 0.02$, both $P < 0.05$) (Fig. 6a and b).

Redundancy analyses (RDA) based on community composition versus environmental and geographical variables showed that in general Patagonian lakes ordinated apart from the Antarctic lakes (with some exceptions for the hypertrophic Antarctic Pingüi Pond and the Patagonian shallow lake Verde) when considering microbial communities from DGGE matrices ($n = 40$ and $n = 14$), total Illumina matrix and different Illumina abundance categories (abundant $\geq 1\%$, uncommon $> 0.1\%$ to $< 1\%$ and rare $\leq 0.1\%$) (Fig. 7). To study the main underlying driver of beta-diversity along the latitudinal gradient we applied variation partitioning and Mantel test (simple and partial) analyses (Table 4). RDA performed on each studied microbial eukaryote community matrix showed that forward selection retained the following variables as relevant: PCNM 1 and PCNM 3 (geographical variables), temperature, conductivity, DOC and lake area (environmental variables) (Table 4). Variation partitioning analyses showed that the amount of variation explained by each type of factor (geographical or environmental alone) varied with the molecular approach (DGGE or Illumina) and the community abundance category studied (Illumina data: abundant, uncommon or rare). Purely geographical and environmental factors explained similar and significant amount of variation on both DGGE ($n = 14$) and abundant Illumina microbial eukaryote communities. However, when moving to less abundant communities (i.e. uncommon or rare) the respective amount of explained variance and the significance faded; only purely geographical descriptors influenced significantly the rare communities (Table 4). In agreement with the results obtained with variance partitioning, Mantel tests showed that the similarity in total and abundant OTU community composition (both from Illumina reads) and in dominant OTU community composition (DGGE band pattern) decreased significantly when the distance among water bodies increased and grew up significantly when the water bodies showed more similar local environmental factors. However, the rare OTU community composition (OTUs $\leq 0.1\%$) only decreased significantly when the distance among water bodies increased and showed an independent effect of environmental parameters (Table 4). On the other hand, both tests did not show the same results with the total communities from Illumina. The Mantel approach showed a significant influence of geographical and environmental descriptors, whereas variation partitioning analysis showed only a higher and significant effect of geographical factors (Table 4).

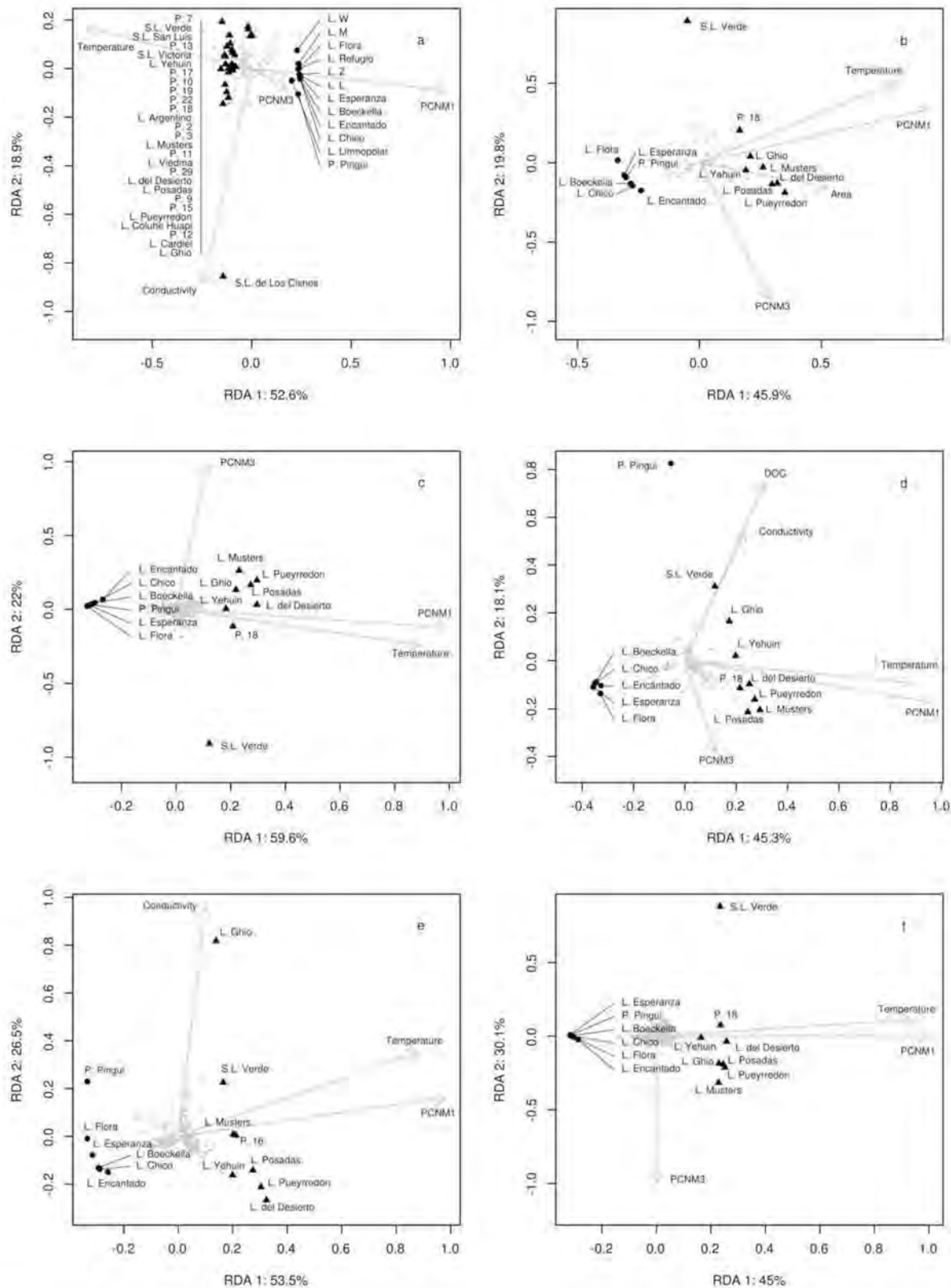


Fig. 7. Redundancy analyses using community composition versus environmental and geographical (PCNM) variables when considering microbial communities from DGGE matrices $n = 40$ (a), $n = 14$ (b), total Illumina matrix (c), abundant $\geq 1\%$ OTUs (d), uncommon $>0.1\%$ to $<1\%$ OTUs (e) and rare $\leq 0.1\%$ OTUs (f). Antarctic lakes are represented by circles and Patagonian lakes by triangles. DOC: dissolved organic carbon.

Table 4. Results of Mantel (simple and partial) and variation partitioning analyses to study the effect of geographical (PCNM) and environmental variables on microbial eukaryote community composition (based on DGGE band pattern and Illumina HiSeq reads).

Matrix type		Mantel		Partial Mantel ^a		Variation partitioning			Forward selection of variables
		<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	Each factor (%)	Both factors (%)	Unexplained (%)	
DGGE (<i>n</i> = 40 lakes)	Geographical	-0.26	0.002	-0.25	0.002	6	5	82	PCNM1, PCNM3
	Environmental	0.15	0.020	0.14	0.020	7			Temperature, conductivity
DGGE (<i>n</i> = 14 lakes)	Geographical	-0.56	0.002	-0.42	0.002	8	8	78	PCNM1, PCNM3
	Environmental	0.47	0.002	0.26	0.020	6			Temperature, lake area
Illumina total OTUs	Geographical	-0.67	0.003	-0.42	0.003	9	17	72	PCNM1, PCNM3,
	Environmental	0.76	0.002	0.60	0.003	2			Temperature
Illumina abundant OTUs ≥1%	Geographical	-0.64	0.002	-0.61	0.002	6	20	66	PCNM1, PCNM3, DOC,
	Environmental	0.57	0.002	0.54	0.002	8			Temperature, conductivity
Illumina uncommon OTUs >0.1 to <1%	Geographical	-0.71	0.002	-0.61	0.002	5	17	73	PCNM1, temperature,
	Environmental	0.60	0.002	0.43	0.002	5			Conductivity
Illumina rare OTUs ≤0.1%	Geographical	-0.71	0.002	-0.63	0.002	9	17	72	PCNM1, PCNM3
	Environmental	0.43	0.004	0.16	0.106	2			Temperature

DOC: dissolved organic carbon.

a. The Partial Mantel test holds geographical or environmental matrix constant. Bold values are significant data ($P < 0.05$).

Discussion

Our results show that microbial eukaryote total richness did not change with latitude (Table 2) and did not differ significantly between Patagonian and Antarctic lakes (Table 1). This contrasts with the prevailing general view of decreasing richness with latitude (Pianka, 1966; Gaston and Blackburn, 2000; Hillebrand, 2004) also observed in soil protists (Lara *et al.*, 2016), although there are a number of exceptions for aquatic (e.g. Crow, 1993; Passy, 2010) and soil (Fernández *et al.*, 2016) eukaryotic microorganisms. Therefore, the decreasing latitudinal gradient in taxa richness often reported for larger organisms (Rosezweig, 1995) is not straightforward for microbial freshwater eukaryotes taken as a whole. Our data are consistent with the report of Hillebrand and Azovsky (2001), which showed that the effect of latitudinal diversity gradient decreases with decreasing body size of the taxa under study and almost disappears for protists (though this paper was based on an incomplete taxonomic sampling for protists). However, when only the abundant fraction of OTUs was considered (from Illumina and DGGE), we observed both a decrease in richness with latitude (Table 2) and significant differences between Patagonian and Antarctic lakes (Table 1). Accordingly, cell abundances (both auto- and heterotrophic) decreased also with latitude and were significantly higher in Patagonia than in Antarctica, probably corresponding to the abundant part of diversity (Tables 1 and 2). Similar decreases with latitude of cell abundances and richness of the dominant taxa were previously observed for bacterioplankton (Schiaffino *et al.*, 2011) and phytoplankton (Izaguirre *et al.*, 2016) in the studied lakes, and for autotrophic picoplankton in the Atlantic Southern Ocean (Doolittle *et al.*, 2008).

The diversity and evenness indices of the whole community did not correlate with latitude (except evenness Pielou index E_H that correlated negatively with latitude, Table 2), whereas both were significantly higher in Patagonian lakes than in Antarctic ones (except E_D , Table 1). As mentioned before, diversity has two basic components, richness (number of OTUs in a community) and evenness (how similar are OTUs in their abundances) (Magurran, 2004), which are combined in diversity indices (e.g. Shannon or Simpson indices). We found that total richness did not change in different latitudes, but as abundance was influenced by latitude (Tables 1 and 2), diversity changed significantly between Patagonian and Antarctic lakes. This pattern of diversity would be due to an increase in harsh climatic conditions with latitude, limiting the development of taxa having lower tolerance to climatic adversity. Protists in high-latitude lakes are constrained by cold temperatures, low inorganic nutrient supply and low light availability for much of the year due to ice cover and polar darkness (Rengefors *et al.*, 2012; Charvet *et al.*, 2014). Basically, many of the OTUs found in Antarctic systems could not reach the threshold to be abundant, likely because these ecophysiological constraints. These could also explain why Patagonian lakes showed more dominant OTUs, whereas Antarctic lakes showed more rare OTUs.

Many authors suggest that richness and evenness should be treated separately (e.g. Hurlbert, 1971; Magurran, 1988; Legendre and Legendre, 1998) because both provide meaningful insights into community function (McNaughton, 1977) and may respond independently to different ecological processes (Ma, 2005). Wilsey and Stirling (2007) proposed that the richness component is more sensitive to migration rates, while evenness is more

sensitive to biotic and environmental interactions. We found that evenness and richness were not correlated along the latitudinal gradient of lakes, suggesting that microbial eukaryote communities responded independently to different ecological factors. As no changes in total OTU richness were found in our dataset, but changes in evenness Pielou index (E_{H}) along the latitudinal gradient of lakes emerged (Tables 1 and 2), we can suppose that microbial eukaryotes would be more regulated by biotic interactions and environmental stressors, than dispersal and migration. Accordingly, Soininen *et al.* (2012) stated that species richness and evenness may respond to different environmental factors or in a different way to a given factor, thus reflecting independent components of biodiversity.

We found a small number (around 8%) of highly abundant OTUs ($\geq 1\%$), representing the 'core biosphere', while most OTUs (around 92%) were less frequent (Fig. 5), the so called 'rare biosphere'. In line with our results, Nolte *et al.* (2010) showed that a small number of highly abundant protistan OTUs are present throughout the entire year, while most OTUs are rare and highly restricted. Similarly, Debroas *et al.* (2015) found that freshwater protists were represented by a high number (77%) of rare OTUs.

Interestingly, we observed differences in taxonomic composition between Patagonian and Antarctic water bodies, being Cryptophyta frequent in Patagonia and absent from Antarctic lakes, whereas Chrysophyta dominated in Antarctic lakes (Fig. 4). This result was also supported by microscopic observations (Izaguirre *et al.*, 2016). Accordingly, Charvet *et al.* (2012; 2014) found that Arctic lakes often contain many OTUs related to Chrysophyta, suggesting they are well adapted to cope with the low nutrient supply and strong seasonality that characterize Polar Regions. Indeed, it has been shown that at least some heterotrophic strains are specifically adapted to the Antarctica (Boenigk *et al.*, 2007).

Multiple-sites dissimilarity measures of variation in OTU composition conducted on Illumina and DGGE dataset revealed that there is a high and significant dissimilarity in the OTU composition between any pair of lakes taken at random from our study site. These analyses also revealed for both datasets that variation in OTU composition is characterized by turnover and to a much lower extent to nestedness (Fig. 6). Most OTUs are replaced among lakes while very few OTUs co-occur among lakes. These results give further support to those shown by the Mantel test and variation partitioning analyses and suggest that each lake may have a unique and little explored microbial eukaryote community. It is worth mentioning that, although a percentage of OTUs is shared between Patagonian and Antarctic lakes (i.e. around 32% of the total Illumina matrix and 70% from DGGE data), turnover remains as an important phenomenon in driving variation in OTUs composition

between any pair of lakes (i.e. OTUs are replaced between lakes). Therefore, these values of shared OTUs between Patagonian and Antarctic lakes should not be confused with the amount of dissimilarity that exists between any pair of lakes in terms of their OTU composition. Indeed, an OTU only needs to be present in one Patagonian lake and in one Antarctic lake to be considered a shared OTU. Moreover, the number of shared OTUs is a descriptive approach that does not say anything about the phenomenon characterising beta-diversity patterns (turnover or nestedness) along the studied gradient.

Mantel test and variation partitioning analyses showed that beta-diversity changed significantly along geographical and environmental gradients (Fig. 4 and Table 4). The distance-based approach Mantel test (that provides an assessment of the significant interactions) and canonical variation partitioning (that allows quantification of the major sources of variation in the dependent variables using transformed raw data) are complementary approaches (Tuomisto and Ruokolainen, 2006). Particularly, these two approaches showed that environmental and geographical factors were important in shaping abundant/dominant microbial eukaryote communities, whereas only the geographical factors were important driving rare microeukaryote communities ($\leq 0.1\%$). These results point towards the existence of an abundant fraction of diversity ('core biosphere') ruled by standard macroecological rules, such as the latitudinal gradient of diversity and environmental filters. It coexists with a 'rare biosphere' that seems to be only weakly influenced by geographical position and not at all by environmental filters. Besides, variation partitioning showed that the percentage of variance explained in the communities decreased in the rarest fractions of diversity, suggesting higher randomness. The combined effect of environmental and geographical factors on the microbial community structure was also found by Cottenie (2005) and Lovejoy and Potvin (2011). Soininen *et al.* (2011) found that freshwater planktonic communities may be controlled by both dispersal-driven assembly and local ecological determinism, with a scale-dependant balance between these two forces. In addition, Lepère *et al.* (2013) and Logares *et al.* (2014) also found a highly significant effect of geographical distance on both rare and abundant/dominant freshwater and marine microbial eukaryotes respectively.

Direct observation of microbial eukaryotes confirmed that autotrophic and heterotrophic eukaryote abundances ($\leq 5 \mu\text{m}$ in diameter) not only showed a latitudinal gradient (Tables 1 and 2), but also their abundances increased with increasing chl *a* (Table 2). Similarly, Rochera *et al.* (2013) found that the abundance of autotrophic picoplankton from 12 lakes of Byers Peninsula increased along a trophic gradient.

It is well known that in all aquatic ecosystems, the sequential filtration process allows the passage of cells

larger than their nominal pore sizes and can lead to the retention of smaller cells if the filters are clogged (Díez *et al.*, 2001). Sørensen *et al.* (2013) found that the abundance of large protist (>10 µm as ciliate and dinoflagellate), often found among picoeukaryotes studies, correlated negatively with the pore size chosen for the end filter in the sequential filtration, suggesting that extracellular DNA adhering to small particles may be the source of larger protist OTUs in picoplanktonic size fractions. We observed also large ciliates by microscopy (such as *Stentor* sp.), which may have contracted and passed through the filters (Gabriela Küppers, personal communication). On the other hand, temporal samplings are important for adequate diversity and species richness estimates (Nolte *et al.*, 2010). It is likely that a broader seasonal sampling would have increased the taxonomic diversity even further and possibly defined better the 'core and rare biosphere'. However, given the considerable amount of sampled lakes and the relatively broad scale studied (2100 km) in our work, we decided to take an integrated water sample in each lake during the same season to make them comparable. Lepère *et al.* (2013) found that temporal variations in the composition of the small protist community do not affect the importance of geographical distance in explaining community composition, and they suggested that the use of a single sample per lake should be enough to analyse the spatial distribution of lacustrine protists.

Although the high-throughput sequencing approach brought obviously more information than DGGE on community composition, comparing both approaches has certain limitations. Indeed, the first PCR step used different primers sets amplifying, respectively, the V9 and V1-3 regions of the SSU rRNA. It has been shown that primers amplifying these regions in eukaryotes are not entirely universal, and therefore, biases may be observed in the respective community compositions (Hadziavdic *et al.*, 2014). Moreover, the taxonomic resolution of the different variable regions of the gene is not equivalent and varies from taxon to taxon (Hu *et al.*, 2015). Nevertheless, in spite of these important methodological differences, a similar amount of variance could be explained with both Illumina abundant OTUs ($\geq 1\%$) and DGGE (Table 4), even though patterns observed in the RDA analyses showed some differences (Fig. 7b and d). In that sense, DGGE gave a picture of diversity comparable with the abundant fraction of the communities, and therefore does not lose its relevance as a fingerprinting technique (Pedrós-Alió, 2006). Our data is consistent with that of Pommier *et al.* (2010), who found that fingerprinting approaches such as DGGE clustered samples in a similar way than a high-throughput sequencing approach (in that case, pyrosequencing).

In summary, we found more abundant OTUs in Patagonian lakes (higher E_H evenness indices and higher dominant/abundant richness) than in Antarctic lakes, while

total OTU richness did not change. Accordingly, the 'core biosphere' was significantly larger in Patagonian lakes than in Antarctic ones, while the 'rare biosphere' was larger in Antarctic lakes. In addition, the microbial eukaryote community composition (beta-diversity) changed significantly between Patagonian and Antarctic lakes and was mainly characterized by OTUs turnover. Both geographical position of the water bodies and the local environmental descriptors influenced beta-diversity (mainly abundant/dominant microbial eukaryote community composition), whereas only the geographical factors were important in shaping the rare OTU community composition. Thus, our results suggest that the latitudinal gradient becomes evident depending on the diversity component under evaluation (i.e. total richness, dominant richness, diversity indices and composition). These findings definitely shed new light on the biogeographical patterns and forces that structure inland microbial eukaryote composition across broad spatial scales. Growing information about microbial eukaryote diversity and distribution patterns makes protist the subject of active arguments over their ecology (Caron *et al.*, 2009). However, microbial eukaryotes comprise an immense array of different sizes and lifestyles, and, even though some general conclusions can be drawn, the study of individual taxa may lead to diverging conclusions. Altogether, patterns in the spatial distribution of organisms provide important baseline information about mechanisms that regulate the diversity of life and the complexity of ecosystems (Levin, 1992; Green *et al.*, 2004).

Experimental procedures

Studied lakes and samplings

Samples were collected once in 40 freshwater bodies (ranging from oligotrophic to eutrophic) from Chubut Province, Argentinean Patagonia, to Hope Bay, Antarctic Peninsula (45°22' to 63°24' S of latitude) (Fig. 1). In Antarctic lakes, samples were taken during the austral summer 2004, whereas in Patagonian lakes, samples were collected in late spring 2007 and 2008. Integrated samples were collected within the euphotic zone from the surface down to 5 m in deep lakes and from about 30 cm below the surface in shallow lakes. Temperature, pH, conductivity and dissolved oxygen (DO) were measured *in situ* with portable meters (Horiba D-54 meter, Kyoto, Japan and Hanna HI 9146, Villafranca, Italy). Nutrients (Ammonium N-NH₄, nitrate N-NO₃, nitrite N-NO₂ and phosphate P-PO₄), phytoplanktonic chlorophyll *a* (chl *a*), dissolved organic carbon (DOC) and light diffuse attenuation coefficient (K_d) were analysed following the methods described in Schiaffino *et al.* (2011).

A complete description of the studied area and the main characteristics of the water bodies were previously reported in different papers: Quirós and Cuch (1985) and Quirós and Drago (1999) for Patagonia, Vinocur and Unrein (2000) for Potter Peninsula, Toro *et al.* (2007) for Byers Peninsula and Izaguirre *et al.* (1998) for the Antarctic Peninsula (Hope Bay).

The geographical positions of the studied water bodies, as well as their main limnological characteristics were detailed in Schiaffino *et al.* (2011). The classification of the trophic status of the water bodies was based mainly on chl *a* taking into account the ranges presented in the Wetzel (2001) compilation.

Quantification of total abundances

Abundances of autotrophic and heterotrophic eukaryotes of picoplanktonic and nanoplanktonic size (0.2–5 µm) from each water body were obtained by epifluorescence, using an Olympus microscope (Olympus BX40F4, Tokyo, Japan) at 1000× magnification. Samples for epifluorescence microscopy were preserved with filtered cold glutaraldehyde 10% (1% final concentration) and filtered through a 0.2 and 0.6 µm pore-size polycarbonate filters. Autotrophic eukaryotes (≤3 µm in diameter) counts were performed using both blue and green-wavelength excitation and DAPI stained heterotrophic eukaryotes (flagellates of ≤5 µm in diameter) counts were performed using blue and UV-wavelength excitation.

Molecular methods

DNA extraction. Around 1000 ml of water samples were pre-filtered *in situ* through a 50 µm net to remove zooplankton, then filtered with a vacuum pump first through a 20 µm pore-size polycarbonate filter and then through a 3 µm and 0.2 µm pore-size polycarbonate filters (diameter 47 mm; Millipore, Cork, Ireland). The filters were placed in cryovials with 1.8 ml of lysis buffer (40 mM EDTA, 50 mM Tris-HCl, 0.75 M sucrose) and stored at –80°C until DNA extraction. Fractionation with 3 µm filters was chosen to be consistent with previous work (e.g. Massana *et al.*, 2004; Vaultot *et al.*, 2008) focusing on microbial eukaryotes. The 0.2–3 µm size fraction was used for this study. The procedures followed for DNA extraction (phenol/chloroform extraction) and touchdown polymerase chain reaction (PCR) amplifications were previously described in detail (Unrein *et al.*, 2005).

DGGE analysis. This approach was performed for a total of 40 Patagonian and Antarctic lakes. Results from this fingerprinting method were presented showing the analysis of the entire dataset ($n = 40$) and the analysis of the 14 ($n = 14$) lakes. To perform DGGE amplifications we used the primers designed by Díez *et al.* (2001) targeting the V1–V3 variable regions of 18S rRNA gene: Euk1F (5'-AAC CTG GTT GAT CCT GCC AGT-3') and Euk516r-GC (5'-ACC AGA CTT GCC CTC C-3') with a 40 bp GC-clamp. DGGE analyses were carried out with a DGGE-2000 system (CBS Scientific Company, Del Mar, CA). The 0.75 mm-thick gels of polyacrylamide (acrylamide:bisacrylamide ratio of 37:5:1) were run at 100 V and 60 °C for 16 h in a linear 35 to 55% denaturant agent gradient (100% denaturant agent was defined as 7 M urea and 40% deionised formamide). The gels were stained for 45 min in 1× TAE buffer with Sybr-Gold nucleic acid stain (Invitrogen, Grand Island, NY) and visualized with UV radiation using a Chemidoc system and the Quantity One software (Bio-Rad, Hercules, CA). Samples were run in 3 DGGE gels and about 800 ng of PCR product from environmental samples were applied to individual lanes in the gels. Between 2 and 4

samples were run in all the DGGEs to allow comparison among the gels; thus, respective positions of individual bands in different gels could be determined. Digitized DGGE images were analysed using the Gel-Pro 4.0 software (National Institutes of Health, Bethesda, MD). Changes in band intensities are likely due to relative changes in the abundances of the corresponding populations. With DGGE approach we obtained the dominant OTUs.

DGGE bands were removed from the gel and resuspended in 20 µl of Milli-Q water. The PCR reamplifications were performed with the original primer set and the PCR products were purified with the QIAquick PCR Purification kit (QIAGEN, Hilden, Germany). The reamplified PCR products were used for a sequencing reaction with the corresponding forward primer in Macrogen Sequencing Service (Macrogen, Republic of Korea). The sequences obtained (around 500 bp) were screened for chimeras with KeyDNATools and then compared with the curated ribosomal eukaryotic database PR² (Guillou *et al.*, 2013). All 18S rRNA gene sequences obtained in this study were deposited in GenBank under accession numbers KC923040–KC923068.

Illumina HiSeq. To further explore the diversity distribution patterns, a subset of 14 water bodies (8 Patagonian and 6 Antarctic lakes) from the entire dataset (40 lakes) were analysed with this technology. We amplified extracted DNA using primers specific to the V9 variable region of the 18S rRNA gene using the protocol as in Amaral-Zettler *et al.* (2009), and adapted after Lara *et al.* (2015). Sequencing was performed by the company Fasteris (Geneva, Switzerland) using Illumina HiSeq 2500 technology; paired end reads were around 200 bp in length.

Quality check (Phred score filtering, trimming of the primers and chimera removal) of the sequences was performed following the pipeline developed by de Vargas *et al.* (2015); Briefly, we kept sequences that passed the filtering based on quality values from the paired fastq file, by evaluating the expected error in a 50 bp sliding window and discarding sequences with more than 1% of error in the worst quality window. Sequences were clustered using SWARM (Mahé *et al.*, 2014). The obtained sequences were then taxonomically classified with the curated ribosomal eukaryotic database PR² (Guillou *et al.*, 2013) using the software GGSearch (McWilliam *et al.*, 2013). Sequences affiliated with Metazoa and Embryophyta were removed from the analysis. Sequences were also aligned against the SILVA bacterial and archaeal database to remove possible prokaryotic OTUs.

Data analyses

The Illumina community matrix was studied considering the total OTUs, the abundant OTUs ≥1% of the total number of sequences in a given sample (Pedrós-Alió, 2006; Fuhrman, 2009) and the less frequent OTUs with two different criteria: uncommon >0.1% to <1% and rare ≤0.1% (Fuhrman, 2009; Vergin *et al.*, 2013). The justification for the use of these thresholds is arbitrary, and lies in studies that indicate that organisms that make ≥1% of the total cell number can be detected with PCR-dependent techniques, while those that comprise ≤0.1% are difficult to retrieve (Muyzer *et al.*, 1993; Casamayor *et al.*, 2000). In order to compare the different

samples, we randomly selected the same number of Illumina sequences from each lake (matching the sample with the lowest number of reads, i.e. 14 437 sequences after primers trimming and quality filtering).

To study the diversity patterns, we calculated two different diversity indices for each sample using the total OTUs from the Illumina data: Shannon's index ($H = -\sum p_i \ln(p_i)$) and Simpson's reciprocal index ($D = 1/\sum p_i^2$), where p_i is the relative abundance of each OTU (n/N). In addition, two different evenness indices were also calculated based on the Simpson's reciprocal index ($E_D = D/S$) and the Shannon's index ($E_H = H'/\ln S$, Pielou index), where S is the OTU richness. To estimate total richness, we computed the Chao 1 index ($\text{Chao } 1 = S_{\text{obs}} + (f_1^2/2f_2)$), where S_{obs} is the observed number of OTUs, f_1 is the number of singleton OTUs (represented by a single Illumina read), and f_2 is the number of doubleton OTUs (Chao, 1984). Furthermore, to determine whether sampling was deep enough to get a reasonable estimate of OTU richness, we performed rarefaction curves with the rarefaction function R package *vegan* v. 2-3, 2. The latitudinal diversity pattern was assessed by correlating (Spearman Rho correlations) the diversity components (richness and evenness) and indices recorded in each lake with latitude. In addition, to compare the responses of the individual diversity components, we performed Spearman Rho correlations between them. Student's *t*-tests were used to compare the differences between Patagonian and Antarctic datasets (abundances, richness, evenness and diversity indices). Some variables were LN-transformed to meet normality and homoscedasticity assumptions.

We computed multiple-sites dissimilarity measures of variation in OTUs composition (Baselga, 2012) based on Illumina and DGGE community matrices to explicitly assess the spatial patterns of beta-diversity and their underlying phenomena (pure turnover, pure nestedness, or a combination of both with one of them prevailing over the other) in the model groups under study. In this approach, beta-diversity (β_{SOR}) is first calculated and then partitioned into its spatial turnover (β_{SIM}) and nestedness-resultant dissimilarity (β_{NES}) components. β_{SOR} and β_{SIM} are represented by the dissimilarity values retrieved through the calculation of Sørensen and Simpson dissimilarity indices respectively; while β_{NES} is computed as the difference between these metrics (*op. cit.*). These metrics range from 0 to 1, where higher values are consistent with higher values of dissimilarity among sites. Differences in the number of lakes among the areas studied were controlled for by re-sampling four lakes from each area 1000 times and computing the average β_{SOR} , β_{SIM} and β_{NES} respectively. Multiple-site dissimilarity measures of beta-diversity were computed using the R package *betapart* (Baselga and Orme, 2012).

We finally determined if environmental parameters and/or geographical distance influenced microbial eukaryote community composition (beta-diversity), and if so, which one had the strongest influence. We also wanted to know if the three abundance categories (abundant $\geq 1\%$, uncommon $>0.1\%$ to $<1\%$ and rare $\leq 0.1\%$) were influenced by the same parameters and if these parameters explained the same amount of variance. We also treated DGGE data in order to evaluate its performance with Illumina data, comparing the patterns using DGGE and the abundant part of the Illumina reads. We first transformed OTU abundance

data using a Hellinger transformation prior to applying redundancy analysis (RDA) (Legendre and Gallagher, 2001; Ramette, 2007). Geographical coordinates were transformed into Principle Coordinates of Neighbourhood Matrix (PCNM; Borcard and Legendre, 2002). Raw environmental variables were transformed to their standard normal deviate equivalents [($x - \text{mean}$) divided by the SD] to accommodate the different units of the different variables (Legendre and Birks, 2012). Relevant variables were selected for each abundance category and DGGE data by forward selection based on RDA (*ordistep* function, R package *vegan* v. 2-3, 2). In order to assess the relative contribution of each factor (environmental vs. geographical descriptors), we performed a variation partitioning based on partial RDA (*varpart* R package *vegan* v. 2-3, 2) on each community matrix (DGGE data and each abundance category from Illumina), also based on the variables that were kept after forward selection. We also performed standard and partial Mantel tests to examine the respective effect of geographical distance versus environmental factors on community composition; this distance-based approach is efficient in analyzing spatial patterns in community similarity and this is an alternative way to assess beta-diversity (Legendre *et al.*, 2005; Tuomisto and Ruokolainen, 2006; Soininen *et al.*, 2007; 2011). We calculated Bray Curtis distance matrices for community matrices (each abundance category from Illumina and DGGE datasets) and Euclidean distance matrices for the standardised environmental parameters (Martiny *et al.*, 2006) and the PCNM variables that were selected previously.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Appendix B. A legacy of human-induced ecosystem changes: spatial processes drive the taxonomic and functional diversities of testate amoebae in *Sphagnum* peatlands of the Galápagos



ORIGINAL
ARTICLE



A legacy of human-induced ecosystem changes: spatial processes drive the taxonomic and functional diversities of testate amoebae in *Sphagnum* peatlands of the Galápagos

Bertrand Fournier^{1,2*}, Emily E. D. Coffey³, W. O. van der Knaap⁴,
Leonardo D. Fernández^{2,5}, Anatoly Bobrov⁶ and Edward A. D. Mitchell^{2,7}

¹Institut des Sciences de l'Évolution UMR5554, Centre National de la Recherche Scientifique, Université Montpellier 2, 34095 Montpellier Cedex 05, France, ²Laboratory of Soil Biology, Institute of Biology, University of Neuchâtel, CH-2000 Neuchâtel, Switzerland, ³Long-term Ecology Laboratory, Biodiversity Institute, Oxford Martin School, Department of Zoology, University of Oxford, Oxford OX1 3PS, UK, ⁴Institute of Plant Sciences and Oeschger Centre for Climate Change Research, University of Bern, CH-3013 Bern, Switzerland, ⁵Laboratorio de Ecología Evolutiva y Filoinformática, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C Concepción, Chile, ⁶Lomonosov Moscow State University, Moscow, Russia, ⁷Jardin Botanique de Neuchâtel, 2000 Neuchâtel, Switzerland

ABSTRACT

Aim Our aims were to compare the composition of testate amoeba (TA) communities from Santa Cruz Island, Galápagos Archipelago, which are likely in existence only as a result of anthropogenic habitat transformation, with similar naturally occurring communities from northern and southern continental peatlands. Additionally, we aimed at assessing the importance of niche-based and dispersal-based processes in determining community composition and taxonomic and functional diversity.

Location The humid highlands of the central island of Santa Cruz, Galápagos Archipelago.

Methods We survey the alpha, beta and gamma taxonomic and functional diversities of TA, and the changes in functional traits along a gradient of wet to dry habitats. We compare the TA community composition, abundance and frequency recorded in the insular peatlands with that recorded in continental peatlands of Northern and Southern Hemispheres. We use generalized linear models to determine how environmental conditions influence taxonomic and functional diversity as well as the mean values of functional traits within communities. We finally apply variance partitioning to assess the relative importance of niche- and dispersal-based processes in determining community composition.

Results TA communities in Santa Cruz Island were different from their Northern Hemisphere and South American counterparts with most genera considered as characteristic for Northern Hemisphere and South American *Sphagnum* peatlands missing or very rare in the Galápagos. Functional traits were most correlated with elevation and site topography and alpha functional diversity to the type of material sampled and site topography. Community composition was more strongly correlated with spatial variables than with environmental ones.

Main conclusions TA communities of the *Sphagnum* peatlands of Santa Cruz Island and the mechanisms shaping these communities contrast with Northern Hemisphere and South American peatlands. Soil moisture was not a strong predictor of community composition most likely because rainfall and clouds provide sufficient moisture. Dispersal limitation was more important than environmental filtering because of the isolation of the insular peatlands from continental ones and the young ecological history of these ecosystems.

Keywords

biodiversity, dispersal, disturbance, environmental filtering, island biogeography, microbial ecology, soil protozoa, functional trait

*Correspondence: Bertrand Fournier, Institut des Sciences de l'Évolution UMR5554, Centre National de la Recherche Scientifique, Université Montpellier 2, CC 065, Place Eugène Bataillon, 34095 Montpellier Cedex 05, France.
E-mail: bfourni@gmail.com

INTRODUCTION

Although the Galápagos Islands are considered to be a conservation hotspot (Coffey *et al.*, 2012), the humid highlands of Santa Cruz Island have undergone profound alterations due to anthropogenic pressure including introduced plant and animal species (Jäger *et al.*, 2009) and because of the hunting, to near extinction, of the giant tortoises that until 500–700 years ago roamed all habitats from the sea shore to the highlands (Froyd *et al.*, 2014). *Sphagnum* mosses, a dominant floristic element of mid- to high-latitude peatlands, are present in the highlands of the four largest islands of the Galápagos archipelago (Isabela, Santa Cruz, Santiago and San Cristóbal) (Itow & Weber, 1974), but *Sphagnum*-dominated

peatlands are known only to persist in the fern–sedge formation on Santa Cruz (Fig. 1).

From a biogeographical point of view, the *Sphagnum* peatlands of the Galápagos represent ‘islands within islands’ of wet and acidic habitats in otherwise arid and neutral to alkaline landscapes (i.e. soils developed from the weathering of lava rocks or uplifted marine sediments). These small peatlands developed mainly in craters at altitudes ranging between 650 and 850 m a.s.l. (Itow & Weber, 1974).

These peatlands also have an unusual history. Recent palaeoecological studies have shown that one of the drivers of the *Sphagnum*-dominated vegetation transition during the last 500–700 years is the near extermination of giant tortoises. Tortoises previously impacted these peatlands by



Figure 1 Top: (a) View of Psidium Bog in the highlands of Santa Cruz Island. The tree fern *Cyathea weatherbyana* and bracken (*Pteridium aquilinum* v. *arachnoideum*) are visible in the foreground; the invasive quinine trees (*Cinchona pubescens*) are mostly dead directly around the peatland thanks to active eradication, but still form a fully developed forest in the distance. (b) Detail of *Sphagnum* peatbog with *Sphagnum perichaetiale*, the dwarf shrub *Pernettya howellii*, and the ferns *Elaphoglossum engelii*, *Nephrolepis cordifolia* and *Dicranopteris flexuosa*. (c) Giant tortoise in a shallow pond at El Chato, Santa Cruz Island. (d) Tortoise faeces. (e) Physical disturbance of the soil caused by adult giant tortoise (half buried in mud in background).

bringing nutrients through their faeces and through physical disturbance (Fig. 1). Many of these *Sphagnum* peatlands were likely historically more minerotrophic ecosystems such as freshwater ponds dominated by aquatic plants (Froyd *et al.*, 2014). Such peatlands have a low diversity of bryophytes; only two *Sphagnum* species have been recorded (*S. perichaetiale* and *S. trinitense*; Froyd *et al.*, 2014). The microtopography typically observed in northern mid- to high-latitude continental peatlands (e.g. hummock-hollow complexes) is absent from the Galápagos.

Although the modern vegetation of these peatlands is well documented, nothing is known of their microbial diversity. The structure, location (in relation to higher latitude peatlands) and the indirect anthropogenic influence on the development of these peatlands make them an interesting model to study the diversity of soil microorganisms, and to assess how different factors have contributed to shaping the communities. Our focus here is on testate amoebae (TA), a group of free-living protozoa known to be abundant and diverse in Northern and Southern Hemispheres mid- to high-latitude peatlands (Wilmschurst *et al.*, 2003; Mitchell *et al.*, 2008; Van Bellen *et al.*, 2014).

We are particularly interested in understanding the assembly mechanisms shaping TA communities as their ecology on the Galápagos peatlands is poorly known. Moreover, understanding these mechanisms can provide information about the functioning of the microbial foodweb and the whole peatland ecosystem. Often, studies examining the relationships between species composition and environmental gradients fail to address the mechanisms responsible for changes in community composition and diversity. Functional traits provide a useful complement to taxonomic approaches of biodiversity as they provide information about competitive interactions and environmental filtering, which in turn allows discriminating between neutral and niche assembly processes. Patterns of functional traits and their associated diversity – functional diversity (FD) – are increasingly studied alongside taxonomic diversity (TD). This approach has up to now been applied to plants (e.g. Janeček *et al.*, 2013) and animals including birds (Meynard *et al.*, 2011), insects (Moretti *et al.*, 2010) and fishes (Villéger *et al.*, 2010). By contrast, functional-trait based approaches are only starting to be applied to microbial taxa (Fournier *et al.*, 2012).

We selected TA as model organisms because: (1) they are the dominant group of protozoa in *Sphagnum*-dominated peatlands (Gilbert *et al.*, 1998), (2) they are top-predators in the microbial food web (Jassey *et al.*, 2013a), (3) they play significant roles in the cycling of elements in terrestrial ecosystems (Aoki *et al.*, 2007) and changes in their communities may thus affect the functioning of ecosystems (Jassey *et al.*, 2013b), (4) they produce shells of diverse shapes and sizes that can be used to define functional traits (Fournier *et al.*, 2012) and (5) their high abundance and diversity allows them to respond fast to environmental changes and they are thus useful bioindicators for monitoring present and past ecosystem changes (Mitchell *et al.*, 2008; Payne, 2013).

Testate amoebae thus are good model organisms to study soil biodiversity while informing on ecosystem functions.

Here, we study the occurrence of TA species on the peatlands of the Galápagos Islands as compared to Northern Hemisphere and South American peatlands. We then investigate the drivers of species distribution using a macroecological perspective. We compare taxonomic and functional-trait patterns of TA across different habitats of Santa Cruz Island (Galápagos) with the aim of assessing the relative importance of niche-based and dispersal processes in determining community composition. Moisture and/or depth to the water table have repeatedly been shown to best explain community patterns in TA in mid- to high-latitude *Sphagnum*-dominated peatlands (Mitchell *et al.*, 2008). Assuming that in *Sphagnum* peatlands environmental controls on community patterns are similar in the Galápagos, we hypothesize that TD and FD will be lower in drier sites and in non-*Sphagnum* habitats. We also hypothesize a high species and functional-trait turnover (beta diversity) among contrasting habitats (i.e. wet versus dry *Sphagnum* and *Sphagnum* versus non-*Sphagnum*). With respect to functional traits, shells of TA in drier conditions are hypothesized to be smaller, more compressed and with a central aperture. In comparison, TA shells in wetter conditions are expected to be larger, less compressed and with a terminal aperture (cf. Fournier *et al.*, 2012; Jassey *et al.*, 2013b).

METHODS

Study site, sampling and microscopic analysis

The study sites are located in the highlands of the central island of Santa Cruz (Galápagos) between 557 and 825 m a.s.l. (~00°38.70' S, 90°19.50' W), in the humid highland climatic zone within the fern-sedge vegetation formation (Trueman & d'Ozouville, 2010). The fern-sedge formation extends from Mount Crocker (864 m a.s.l.), the highest point on Santa Cruz, to approximately 570 m a.s.l. The highlands of the southern slope of Santa Cruz are often enveloped in clouds and fog and receive ca. six times more precipitation than the coastal zone (i.e. ca. 1803 mm in Media Luna at 620 m a.s.l. vs. 306 mm at the Charles Darwin Research Station at 6 m a.s.l.) (Itow & Weber, 1974).

A total of 51 samples (uppermost ca. 5 cm of moss, litter or soil) were collected from eight sampling sites (see Table S1 in Appendix S1 in Supporting Information). Six sites were *Sphagnum*-dominated peatlands located in six different cinder cone craters (i.e. closed basins with no perennial streams). Five of these craters had well-developed *Sphagnum* peatlands (Fig. 1), while the sixth, comparatively shallow crater had only smaller patches of *Sphagnum*-dominated vegetation (i.e. 'Inconspicuous Bog', ca. 100 m² peatland). The two remaining sites were located outside craters in the upland areas, in temporarily wet patches on the side of trails or valley-bottom fens. The samples encompassed the full gradient of wet to dry habitat including *Sphagnum* bogs,

ephemeral pools, mosses, soil litter, and trails. At each site, the broadest available range of micro-habitats was sampled, including the contact zone of the peatlands with the mineral soil.

Coordinates and elevation of each sampling point were measured using a hand-held GPS device. Each sample was assigned to a moisture class: FI – submerged mosses; FII – free-floating mosses; FIII – very wet; FIV – wet; FV – semi-wet; FVI – moist; FVII – semi-dry; FVIII – dry (Jung, 1936). Depth to water-table (DWT) was measured in 2 cm diameter holes after a time of *ca.* 30' to allow the water level to stabilize, and ranged from 0 to 79 cm, where zero was defined as the top of the sampling surface. A categorical variable for site topography was created based on the macro-topography of each site as follows: (1) flat ground to slight depressions (side of trails, broad crater with low rims), (2) intermediate topography and craters open on one side and (3) deepest craters (slopes 30% or more, 30 m or more elevation difference between crater ridge and bottom).

Testate amoebae were extracted from 1 g of dried sample. The material was dispersed in 250 ml of distilled water for 24 h, shaken vigorously, sieved over a 250 µm mesh screen, left to settle overnight, and the supernatant was removed. Samples were stored in sealed vials in distilled water with 2% glutaraldehyde. A total of 150 individuals per sample were identified (Mazei & Tsyganov, 2006) and counted at 200× and 400× magnifications. Dead, living, and encysted individuals were not tallied separately.

Testate amoeba communities from the insular peatlands of the Galápagos versus continental peatlands of the Northern and Southern Hemispheres

We compared the average abundance and frequency of testate amoeba genera from *Sphagnum* samples of the Galápagos to databases representative of the mid- to high-latitude Northern Hemisphere and continental South America. The Northern Hemisphere database included 345 samples from Europe and Alaska previously used to document the diversity of peatland TA (Gilbert & Mitchell, 2006). The South America database included 112 samples from Chile and 21 samples from Argentina (Zapata, 2005; Zapata & Fernández, 2008; Fernández & Zapata, 2011; Fernández *et al.*, 2015). The goal was to determine if some taxa were more or less abundant (or altogether absent) in either data set.

Functional traits

We selected six functional traits: pseudopod morphology; origin of the shell material; aperture position; test shape; compression and biovolume as follows:

1. Pseudopod morphology: filose or lobose. These equal two of the three phylogenetic groups distinguished in TA (Meisterfeld, 2002a,b; Nikolaev *et al.*, 2005; Gomaa *et al.*, 2013): Euglyphida that have filose pseudopods and Arcellinida that

have lobose pseudopods. Euglyphida include mainly smaller, bacteria-feeding taxa and are generally considered as *r*-strategists while Arcellinida include both smaller and larger taxa and are generally considered as *K*-strategists. The ratio lobose/filose has been shown to increase from early to late soil developmental stages and is thus used to define the maturity of ecosystems (Bonnet, 1964).

2. Origin of shell 'test' material: agglutinate or autogenous. Testate amoebae either agglutinate material from the surrounding environment or autogenously secrete siliceous or proteinaceous tests (Meisterfeld, 2002a,b). The availability of building material and/or the higher cost of self-secretion can constrain species distribution.

3. Aperture position: central, sub-terminal or terminal. The position of the aperture is believed to represent an adaptation to moisture, where an exposed, terminal aperture is better adapted to wetter conditions, whereas a central, hidden aperture is better adapted to drier conditions (Bonnet, 1964).

4. Test shape: ovoid, cylindrical-ovoid, saucer- or disc-shaped or spherical. Test shape relates to the ability to move in a water film: ovoid shapes allow better movement in thinner water films than spherical shapes. Taxa with spherical tests are thus more likely to be found in wet soils (Bonnet, 1964).

5. Test compression: compressed or non-compressed. Compressed tests are believed to be better suited to live in low moisture conditions with a thin water film, but at a higher cost (more shell material compared to cell biomass), while non-compressed tests (spherical or round in cross-section) may be adapted to very wet conditions (Bonnet, 1964).

6. Biovolume (µm³). Larger species have longer generation times than smaller species and recover more slowly from perturbations, and thus can be viewed as *K*-strategists following Grime (1977). Calculations of biovolume are derived from Fournier *et al.* (2012).

Diversity indices

We calculated alpha, beta and gamma taxonomic diversity (TD) and functional diversity (FD) using Rao partitioning of diversity followed by Jost correction (Jost, 2007; de Bello *et al.*, 2010). Under this definition alpha diversity is calculated as follows:

$$Q_\alpha = \frac{1}{(1 - \sum d_{ij} p_i p_j)} \quad (1)$$

where d_{ij} is the distance between species i and j , p_i and p_j are the relative abundance of species i and j . When measuring FD, d_{ij} is the functional distance between pairs of species. In our case, d_{ij} was calculated using Gower distance to allow for different types of variables. For TD, a distance of 1 is attributed to all pairs of species. Under this definition, α_{Rao} is identical to the Simpson diversity index. Gamma diversity is calculated with the same formula by pooling all local communities. Beta diversity is defined as the difference between

gamma diversity and the mean alpha diversity of all local communities. The advantage of these indices above others is that they provide a common mathematical framework to estimate TD and FD at various spatial scales.

Numerical analyses

We assessed the correlation between environmental variables and community-weighted means of functional traits (CWM) using generalized linear models (GLM). We then used variance partitioning, a technique based on partial redundancy analysis (RDA), to assess the extent to which environmental and spatial variables explain the composition of communities in terms of species and functional traits. This approach can help estimate the relative roles of niche-based and neutral processes and the type of coexistence mechanisms (Cottenie, 2005). Species data were Hellinger-transformed prior to analysis (Legendre & Gallagher, 2001), and CWM data were normalized by subtracting the mean and dividing by the standard deviation. All analyses were performed in R-3.1.2 (R Development Core Team, 2012), package 'FD' (Laliberté & Shipley, 2010).

RESULTS

Testate amoeba community composition

A total of 44 testate amoeba morpho-taxa from 15 genera were identified (see Table S2 in Appendix S1). The most common and overall abundant species were *Trinema enchelys* (relative abundance 12.6%; relative occurrence 88%), *Assulina scandinavica* (11.0%; 63%) and *Cyclopyxis eurytoma* (6.9%; 57%). Euglyphida were present in all samples, represented by five genera and 19 species and infra-specific taxa, and contributing to 56.3% of the overall count with a range of 4.7–100%. Three genera together accounted for over 50% of the total testate amoeba community: *Trinema* (19.1%), *Assulina* (17.3%) and *Euglypha* (14.7%). Arcellinida were represented by 10 genera and 25 species and infra-specific taxa, and accounted for 43.7% of the overall count (range: 0–95.3%). *Cyclopyxis* accounted for 22.2% of the total count and *Quadrullella* 7.4%.

Species with cylindrical-ovoid or spherical tests were more numerous (16 and 12 respectively) than species with ovoid or saucer- or disc-shaped tests (7 and 9 respectively). Species with non-compressed shells were more frequent (25 species). Twenty-five species had a central aperture, whereas 11 and 10 species had a sub-terminal and terminal aperture respectively. Agglutinate and autogenous shell material were encountered in 21 and 23 species respectively. Average biovolume was about $1.3 \times 10^5 \mu\text{m}^3$ and ranged from 1.7×10^3 to $1.4 \times 10^6 \mu\text{m}^3$.

The comparison of relative abundance and frequency of testate amoeba genera in the Galápagos with a compilation of 345 samples from Europe and Alaska and 133 samples from Chile and Argentina revealed some clear contrasts (Fig. 2). Genera common in both Northern and Southern Hemisphere *Sphagnum* peatlands but absent in Galápagos *Sphagnum* peatlands included *Hyalosphenia*, *Bullinularia*, *Heleopera*, *Diffu-*

gia, *Pseudodiffugia* and *Tracheleuglypha*, whereas *Nebela s.l.* is strongly under-represented in Galápagos. *Archerella* and *Physochila* were only found in the Northern Hemisphere peatlands and *Alocodera*, *Apodera*, *Certesella*, *Cyphoderia*, *Lesquereusia* and *Schwabia* only in the Southern American continental peatlands (the latter three taxa are known from the Northern Hemisphere but their ecological optima are not in *Sphagnum*). In general mixotrophic species (*Archerella*, *Amphitrema*, *Hyalosphenia papilio*, *Placocista spinosa*, *Heleopera sphagni*) were absent from the Galápagos samples. By contrast the Galápagos samples contained a much higher average relative abundance of *Assulina*, *Cyclopyxis*, *Quadrullella* and *Trinema* and higher frequency of *Cyclopyxis*, *Trigonopyxis*, *Plagiopyxis*, *Quadrullella* and *Trinema*.

Taxonomic and functional diversity

Functional diversity was low as compared to TD (Fig. 3a). The turnover (beta diversity) of species was high and accounted for 72% of taxonomic gamma diversity, which strongly contrasts with the 12% observed for FD (Fig. 3b).

Species richness and alpha TD were not significantly correlated with environmental variables (Table 1). However, we observed a weak but significant increase of alpha FD at sites located in closed and deep craters, where *Sphagnum* was most abundant. Changes in community-weighted mean of functional traits among sites were low and only weakly correlated with environmental variables. Moisture, DWT, material, and habitat were not correlated with functional-trait distribution. Nevertheless, species with filose pseudopods and large spherical shells were more abundant at higher elevation, while species with non-compressed shells were more abundant in the deepest and most closed craters, where ambient moisture was highest.

Composition and functional patterns of testate amoeba communities

The species and functional-trait composition of testate amoeba communities was more strongly correlated with spatial than with environmental variables (Fig. 4). Overall spatial variables explained 30% and 67% of the variance in species and functional-trait data respectively, whereas environmental variables explained 9% and 19% respectively. Moreover, the partial RDA revealed that space alone significantly explained 28% of the variance in taxonomic diversity and 48% in functional diversity, while the correlation with environment alone was not significant.

DISCUSSION

Diversity of testate amoebae in the Galápagos peatlands

This is the first ecological study on TA and only the third report of these organisms in the Galápagos. Ehrenberg

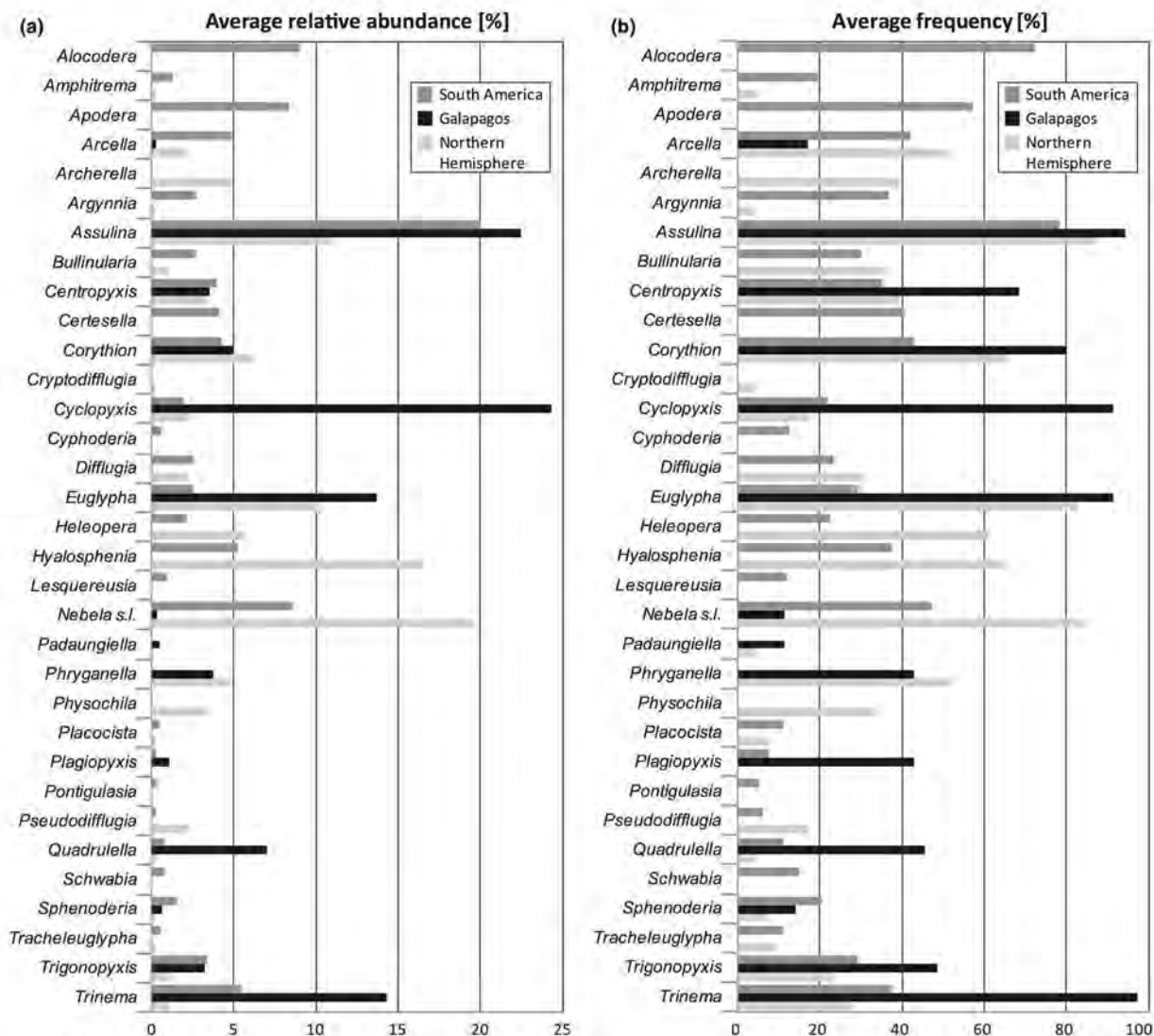


Figure 2 Average relative abundance (a) and frequency (b) of occurrence of testate amoeba genera in *Sphagnum* samples from peatlands in the Galápagos Islands as compared to 345 samples from continental peatlands in Europe and Alaska (Northern Hemisphere) and 133 samples from continental peatlands in Chile and Argentina (South America).

(1853) found 12 taxa in 15 samples sent to him by Darwin. Decloître (1973) analysed seven samples from Santa Cruz. He reported 62 taxa, among which 14 were new to science. He noted that *Diffugia* was absent (but it was listed by Ehrenberg), *Arcella* and *Nebela* were represented by only a few taxa, but *Trinema* was abundant and especially diverse. We identified all observed individuals to species level and found no taxa endemic to the Galápagos (see Table S2 in Appendix S1). Among our 44 taxa, 26 represent new records, 18 were previously reported by Decloître and one by both Ehrenberg and Decloître (see Table S2 in Appendix S1). The low overlap in species composition between the studies is not surprising, given that Decloître's samples were from habitats very different from ours (cultivated soil, *Scalesia* forest soil, mosses growing on trees and rocks, ponds). Never-

theless, our results agree in that *Trinema* is abundant (19.1% of the overall count) and diverse (4 taxa, compared to 12 by Decloître). The overall list of TA recorded to date amounts up to 86 taxa.

The generic composition recorded in our insular peatlands is only an impoverished subset of that recorded in continental peatlands. Genera like *Diffugia* and *Hyalosphenia* are abundant and frequent in all continental peatlands (Mitchell *et al.*, 2008) but were absent in the Galápagos. Usually these genera include large species (> 100 µm) (Meisterfeld, 2002a); thus, the probability of these having been overlooked in our insular samples is low. Likewise, several distinctive taxa of South American continental peatlands such as *Alocodera* and *Certesella* were also absent. Although these taxa are generally not abundant in South American peatlands (Zapata, 2005;

Fernández & Zapata, 2011), they always are easily found in samples due to their conspicuous sizes (Zapata & Fernández, 2008). In addition, shared genera exhibited different abundances and frequencies between insular and continental peatlands. *Cyclopyxis* and *Trinema* are infrequent taxa in continental peatlands (Gilbert & Mitchell, 2006), but they dominated in the Galápagos. In contrast, *Arcella* and *Nebela* exhibit high abundances and frequencies in continental peatlands (Zapata, 2005), but they appeared as scarce and unusual taxa in the Galápagos. Overall, our results suggest that the TA with the largest tests were underrepresented in the Galápagos *Sphagnum* peatlands, whereas the genera exhibiting high abundances and frequencies were often generalists, such as *Cyclopyxis* and *Trinema*.

The remoteness and the small area of the island most likely explain the differences in TA species composition,

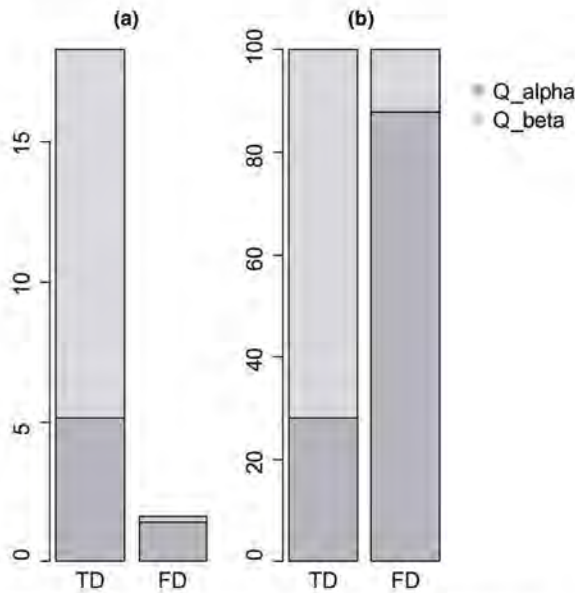


Figure 3 Absolute (a) and standardized (b) partitioning of testate amoeba Rao (Q) taxonomic (TD) and functional diversity (FD) into alpha, beta and gamma components in peatland habitats of the Galápagos Islands.

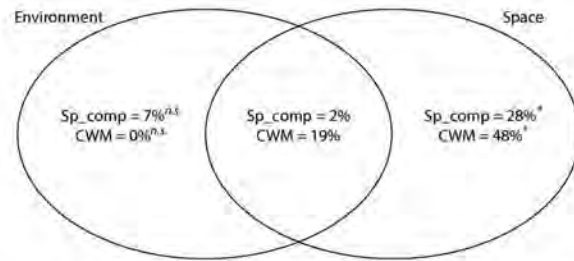


Figure 4 Percentage of variance in testate amoeba community (Sp_comp) and community-weighted means of functional traits (CWM) explained by environmental (left circle) and spatial (right circle) variables in peatlands of the Galápagos Islands.

abundance and frequency between the island and continental peatlands. According to the theory of island biogeography (MacArthur & Wilson, 1967), the diversity of taxa decreases with the isolation of the island, and increases with island area. Thus, the generic-level composition in the peatlands of Santa Cruz Island should be an impoverished subset of that recorded in their continental counterparts because (1) only those taxa that are good dispersers may colonize it, and (2) their area represents only a small fraction of all continental peatlands surveyed. However, while the area *per se* probably does not directly determine the number of protist taxa that are present in the insular peatland, it probably determines the heterogeneity of this habitat (e.g. pH, temperature and moisture ranges) and thus, the number of inter-specific interactions that occur there (Hutchinson, 1957; Allouche *et al.*, 2012).

Besides the above predictions, the airborne dispersal hypothesis (Wilkinson, 2001) predicts that the small species (those having a size of below 100 μm) are more likely to be passively dispersed over long distances than large species. In addition, larger taxa may be less likely to form resistant cysts, (Fernández *et al.*, 2012) and *Archerella* was never observed encysted. Such physiological traits may further limit the dispersal potential of peatland TA. Physical and/or physiological aspects may thus explain why all large continental taxa are either absent (e.g. *Apodera*) or present in very small

Table 1 Summary of generalized linear model (GLM) results showing the influence of environmental variables on the community-weighted means of testate amoeba functional traits. Adjusted R^2 , AIC and variable coefficients are given when significant ($\alpha < 0.05$).

	Adjusted R^2	AIC	Elevation	Moisture	DWT	Material	Habitat	Topography
Phylogeny	0.05	6.74	−0.002	ns	ns	ns	ns	ns
Shape	0.19	76.17	−0.003	ns	ns	ns	ns	ns
Compression	0.06	−9.07	ns	ns	ns	ns	ns	0.1
Aperture position	—	—	ns	ns	ns	ns	ns	ns
Material	—	—	ns	ns	ns	ns	ns	ns
Biovolume	0.17	74.77	0.004	ns	ns	ns	ns	ns
Species richness	—	—	ns	ns	ns	ns	ns	ns
Alpha TD	—	—	ns	ns	ns	ns	ns	ns
Alpha FD	0.13	−60.82	ns	ns	ns	0.1	ns	0.06

AIC, Akaike information criterion; ns, not significant; DWT, depth to water-table (cm); TD, taxonomic diversity; FD, functional diversity.

abundances and frequencies (e.g. *Nebela*) in the island. This pattern has also been recorded in other oceanic Pacific islands (Zapata & Crespo, 1990) while experimental island biogeographical approaches confirmed the importance of dispersal for the distribution of ectomycorrhizal fungi (Peay *et al.*, 2012). However, the relative small size of the taxa observed in the peatlands is not sufficient to explain the differing community compositions. For instance, smaller taxa such as those in the genera *Cryptodiffugia*, *Pseudodiffugia* and *Tracheleuglypha*, as well as *Diffugia pulex* and *Hyalosphenia minuta* were lacking on the island but are present in the Northern and/or Southern Hemisphere continental sites. Taxa such as mushrooms, mosses and ferns, with smaller propagule size than TA, can show restricted distributions (Foissner, 2007).

Following the species succession theory, the relatively young ecological history of the peatlands can explain the lack of larger hydrophilous species and high abundance of small generalists. It can be argued that the peatlands are in an early successional stage following the possible extinction of the giant tortoise from the highlands (Froyd *et al.*, 2014). Large hydrophilous species are *K*-strategists (Bonnet, 1964) that are likely to appear in later successional stages. In contrast, small generalists are *r*-strategists (Bonnet, 1964) that are more abundant early in the succession. However, we did not find empty shells that would have indicated that large hydrophilous species were present on the island but unable to establish. This provides further support to the hypothesis of dispersal limitation.

Community–environment relationships

Our results suggest that TA taxonomic and functional-trait composition within the Galápagos *Sphagnum* peatlands are not strongly correlated with DWT, contrary to our hypotheses that were based on the relationships commonly found in mid- to high-latitude Northern Hemisphere peatlands (Mitchell *et al.*, 2008) and in Patagonian peatlands (Van Belen *et al.*, 2014). Instead, our results suggest that species had ovoid tests of larger biovolume at higher elevation where, in addition to high rainfall, an important part of the moisture comes from the inversion layer (Itow & Weber, 1974), and that shells were less compressed in deeper craters, where ambient moisture was highest. This result contrasts with other studies of TA that show a strong relation to DWT at small (Fournier *et al.*, 2012) and global scales (Bates *et al.*, 2013). A potential explanation for this result is the absence of larger hydrophilous species from the Galápagos Islands. However, a strong correlation of species distribution with DWT was observed in soil communities that typically lack the larger hydrophilous species commonly found in peatlands (Fournier *et al.*, 2012). We therefore propose that the ambient (air) moisture is sufficiently high for TA not to depend on soil moisture. This might change in the future with the colonization of the island by large hydrophilous species.

Importance of niche- and dispersal-based processes

Our results suggest that dispersal limitation within the island is more important for TA communities than environmental filtering. This is indicated by the weak correlations of environmental variables with the various components of diversity. Moreover, following Cottenie (2005) our partitioning results can be interpreted as either neutral dynamics or competition–colonization trade-offs, which further confirms the importance of dispersal. Beisner *et al.* (2006) studied the relative importance of spatial versus environmental processes in various lakes. They showed that taxa with relatively low dispersal capacity (fishes, crustaceans and zoo-plankton) are better predicted by spatial than environmental variables, whereas the opposite was observed in taxa with higher dispersal capacity (bacteria and phytoplankton). Hájek *et al.* (2011) found that propagule size can predict the relative importance of spatial versus environmental processes. Our results agree with this, as TA have a lower dispersal capacity and larger propagule size than other microbial taxa (Wilkinson *et al.*, 2012). Strong environmental filtering on TA taxa had previously been demonstrated only at smaller spatial scales and/or in more disturbed environments (Fournier *et al.*, 2012; Jassey *et al.*, 2013b).

Conclusions

Our results show that the testate amoeba communities of the *Sphagnum* peatlands of the Galápagos and the mechanisms shaping these communities contrast with Northern Hemisphere continental peatlands as well as with southern South American continental peatlands. Soil moisture did not emerge as a strong predicting factor for testate amoeba communities most likely because large hydrophilous species that are more sensitive to soil moisture have not yet colonized the insular peatlands and because of the high rainfall and the moisture brought by clouds. Dispersal limitations explain the distribution of TA better than environmental filtering, because the peatlands are isolated from each other and from the continent, and because their ecological history is recent. *Sphagnum* peatlands are currently only known to exist on Santa Cruz, where their development resulted in important local transformations including the (near-) extinction of plant taxa (van Leeuwen *et al.*, 2008; Coffey *et al.*, 2012). Furthermore, it is unclear to what extent global change is likely to further modify them and what will be the consequences of these changes at larger scales (e.g. humid highlands or entire islands). Finally, these peatlands provide a record of past climatic conditions in the Galápagos through pollen and testate amoeba shell records that can be found in the accumulating peat (Coffey *et al.*, 2012).

Future studies should investigate the degree of genetic distance between Galápagos TA and the populations of Northern Hemisphere continental peatlands as well as those occurring on the South American continent to assess the ori-

gin of Galapagan TA, as has been done with better documented taxa such as marine iguanas (Rassmann *et al.*, 1997) or mockingbirds (Arbogast *et al.*, 2006). Assessing whether other microbial groups follow the patterns we observed for TA can inform about how general our results are. Finally, it would be interesting to see if the central functional role of TA inferred from microbial community analyses in Northern Hemisphere bogs (Jassey *et al.*, 2013b) also can be verified in the Galápagos.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Environmental conditions in the sampled plots.

Appendix S2 Testate amoeba species list and functional traits.

BIOSKETCH

The authors are experts in different aspects of the biology of testate amoebae. The research interest of this team is to investigate the taxonomic and functional components of the biodiversity of these organisms on the peatlands of the Galápagos Islands.

Author contributions: B.F., E.E.D.C., W.O.vdK. and E.A.D.M. conceived the ideas; E.E.D.C., W.O.vdK., E.A.D.M. and L.D.F. collected the data; E.E.D.C., A.B., E.A.D.M. and L.D.F. conducted species-level identifications, B.F. and E.A.D.M. analysed the data and all authors contributed to the writing.

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Appendix C. Cinderella's helping pigeons of the microbial world: The potential of testate amoebae for identifying cryptotephra

Cinderella's helping pigeons of the microbial world: The potential of testate amoebae for identifying cryptotephra

Maxence Delaine^{a,b,*,1}, Leonardo D. Fernández^{c,d,*,1}, Eric Armynot du Châtelet^b,
Philippe Recourt^b, Jean-Luc Potdevin^b, Edward A.D. Mitchell^{c,e}, Nadine Bernard^{a,f}

^aLaboratoire Chrono-Environnement, UMR 6249, Université de Bourgogne Franche-Comté, 16 route de Gray, 25030 Besançon Cedex, France

^bUniversité de Lille, UMR LOG 8187 CNRS, ULCO, Laboratoire d'Océanologie et Géosciences, Bâtiment SN5, 59655 Villeneuve-d'Ascq, France

^cLaboratory of Soil Biodiversity, Institute of Biology, University of Neuchâtel, Rue Emile Argand 11, CH-2000 Neuchâtel, Switzerland

^dLaboratorio de Ecología Evolutiva y Filoinformática, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario s/n, Casilla 160-C, Concepción, Chile

^eBotanical Garden of Neuchâtel, Chemin du Perthuis-du-Sault 58, CH-2000 Neuchâtel, Switzerland

^fLaboratoire THÉMA, UMR 6049, CNRS, Université de Bourgogne Franche-Comté, 32 rue Mègevand, 25030 Besançon Cedex, France

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Abstract

Cryptotephra (particles <125 µm) is a key record for monitoring past and current volcanic activity. However, its extraction from the host sediment and analysis is often long and difficult because of its small size. Finding a simple method to extract cryptotephra from environmental samples would therefore make its analysis much easier. We hypothesized that arcellinid testate amoebae may hold such a potential. These free-living shelled protists are among the earliest microorganisms to colonize volcanic tephra, and build their shell by agglutinating minerals from their environment. We analyzed by X-ray Spectrometry the mineral signature of tephra from the 2011 Puyehue-Cordon Caulle Volcanic Complex (Chile) eruption ash fallout and compared it to that of the shells of 51 individual testate amoebae (three individuals from each of 17 species) from 13 samples collected at different distances from the active vent. The mineral composition of particles within shells closely matched that of similar size class particles from their environment. The capacity of testate amoebae to randomly use mineral grains from their environment makes it possible to use their shells to assess the mineral composition of cryptotephra from soil, peat or sediment samples. Testate amoebae therefore represent the microbial world's version of Cinderella's helping pigeons.

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Keywords: Arcellinida; Cryptotephra; Elemental analyzes; Free-living unicellular eukaryotes; Puyehue-Cordon Caulle volcanic complex; Shell composition

* Corresponding author at: Laboratoire Chrono-Environnement, UMR 6249, Université de Bourgogne Franche-Comté, 16 route de Gray, 25030 Besançon Cedex, France.

** Corresponding author at: Laboratory of Soil Biodiversity, Institute of Biology, University of Neuchâtel, Rue Emile Argand 11, CH-2000 Neuchâtel, Switzerland.

E-mail addresses: maxence.delaine@gmx.fr (M. Delaine), limnoleo@gmail.com (L.D. Fernández).

¹ These authors are co-first authors and contributed equally to the paper.

Introduction

In Jacob and Wilhelm Grimm's version of the Cinderella fairy tale (Grimm brothers, 1812) pigeons help Cinderella sort out lentils, seeds and peas from cinders, thus allowing her first to see her evil stepsisters dance at the ball and then to attend the ball herself (with additionally magical tricks of course). This is of course a fairy tale, but have all scientists not dreamt having magical helping hands to speed-up impossible chores? Here we present such a case from the analysis of cryptotephra from terrestrial habitats in which testate amoebae, a group of microorganisms may indeed play the role of a miniature version of Cinderella's pigeons.

Testate amoebae are a species-rich and polyphyletic group of heterotrophic and mixotrophic microorganisms that belong to two deep and distantly related lineages, i.e. Amoebozoa (lobose testate amoebae) and the supergroup SAR (filose testate amoebae) (Adl et al. 2012). These organisms occur in almost all freshwater and terrestrial biomes of the planet, including those as extreme as deserts (Fernández 2015), tundra (Mazei and Chernyshov, 2011) and polar freshwater (Zapata and Matamala 1987); though they are always more diverse in biomes dominated by high yearly precipitation and warm temperatures such as tropical and temperate rainforests (Bamforth 2010; Fernández et al. 2012; Krashevskaya et al. 2007). They also represent one of the most diverse and abundant groups of eukaryotic microorganisms in peatlands, and therefore, their diversity and ecology have been best studied in these habitats (e.g. Bobrov et al. 2002; Charman 1997; Fernández and Zapata 2011; Fournier et al. 2016; Marcisz et al. 2014; Qin et al. 2012).

One of the most conspicuous morphological features of testate amoebae is their proteinaceous or agglutinated shell, or test, which surrounds and protects their single-cell bodies. Proteinaceous shells are produced by the organism itself and can be flexible, rigid or areolated (Meisterfeld, 2002a). By contrast, agglutinated shells are constructed by attaching either self-produced building units (idiosomes) that resemble scales, plates or rods (i.e. idiosomic testate amoebae) or foreign building units (xenosomes) such as mineral particles and/or idiosomes from other testate amoebae (i.e. xenosomic testate amoebae) (Meisterfeld 2002a, 2002b).

The shells of these protists (especially those built by xenosomic testate amoebae) remain intact in peats and sediments for long periods of time after the death of the organism and can be identified to species level in most cases (Beyens and Meisterfeld 2001). Indeed, sub-fossils of these organisms are abundant in Holocene peats and sediments (Charman 2001; Swindles et al. 2010; Wall et al. 2010) and fossils have been identified from sediments and amber as old as the Triassic (220–230 Ma, Schönborn et al. 1999) and even the Neoproterozoic (~750 Ma, Porter et al. 2003).

Testate amoeba communities are known to vary along environmental gradients (Beyens and Meisterfeld 2001; Fernández et al. 2016; Marcisz et al. 2014). This feature coupled with the good preservation of their shell has led to their

use as proxies for monitoring changes in present (Meyer et al. 2012; Turner and Swindles 2012; Valentine et al. 2013) and past (Delaine et al. 2014; Marcisz et al. 2015; Mitchell et al. 2008) environmental conditions. More recently, their usefulness as proxies of volcanic impacts on peatland microbial communities have also been shown (Payne 2012), opening a new and so far little explored practical application for these microorganisms.

Volcanic eruptions are one of the most impressive natural disturbances on Earth. Many volcanic eruptions have occurred throughout Earth's history, with important consequences on the environment and biodiversity (Johansen et al. 1981; Marske et al. 2007; Schoene et al. 2015). Contemporaneous volcanic eruptions have negative impacts on the economy and societies. Volcanic ash fallout (tephra) affects agriculture, livestock, water distribution systems and ground and air transportation network, with major consequences on the farming and tourism sectors (Collini et al. 2013; Folch et al. 2008). Because of this, the constant monitoring of volcanoes and the determination of the frequency, duration and effect of past and current volcanic eruptions have become priority issues in regions where active volcanoes occur (Buteler et al. 2011; Caneiro et al. 2011; Collini et al. 2013; Folch et al. 2008; Lavallée et al. 2008).

The analysis of tephra layers in conjunction with proximal records of volcanic activity is commonly used to deduce information about volcanic ash cloud fallout and frequency (Lane et al. 2014; Watson et al. 2015). More recently, paleoenvironmental studies have also demonstrated the potential of extending the use of visible tephra layers by looking for non-visible volcanic ash horizons known as cryptotephra, i.e. the tephra fraction with a grain size below 125 µm (Blockley et al. 2012; Lane et al. 2014). Unlike visible tephra, cryptotephra layers can be found more than 1000 km from their source. Therefore, cryptotephra analysis allows increasing the area in which tephra layers can be used to date and correlate paleoenvironmental archives (reviewed in Lane et al. 2014).

A problem with using tephra layers for practical purposes is that they may be potentially modified or removed by taphonomic processes (Payne et al. 2005), resulting in an uneven record for these time synchronous marker horizons (i.e. isochrones) (Blockley et al. 2012; Lane et al. 2014). Furthermore, as cryptotephra is composed of microscopic mineral particles, its extraction from the host sediment can be a tiresome and laborious task (Lane et al. 2014). In these cases, the use of complementary proxies could be very useful in facilitating the analysis of visible tephra and filling records gaps by allowing the study of non-visible tephra.

Xenosomic testate amoebae represent potentially useful proxies to fulfill this task. Qualitative and quantitative analyses have shown that testate amoebae incorporate into their shell only the mineral particles available in their immediate environment (Armynot du Châtelet et al. 2010, 2015; Štěpánek, 1952). They are also among the earliest protists to appear in the microbial succession occurring in volcanic

tephra (Smith 1985), and their subfossil and contemporary communities are evenly found in soil and peat tephra deposits (Payne 2012; Smith 1974, 1985). This background led us to hypothesize that xenosomic testate amoebae use tephra to build their shell in sites recently affected by volcanic eruptions. If true, this could lead to establishing xenosomic testate amoebae as reliable proxies of the frequency and extent of past and present-day volcanic eruptions, either alone or as a complement of other approaches. To test this hypothesis, in this study, we conducted elemental and multivariate analyzes to determine and compare the mineral composition of tephra and xenosomic testate amoeba shells in a site affected by the recent eruption of the Puyehue-Cordón Caulle volcanic complex, southern Chile.

Material and Methods

Geographical setting

Chile is a narrow and long country situated in the southeastern Pacific coast. This country has nearly 90 active volcanoes, one of which is the so-called Puyehue-Cordón Caulle volcanic complex (PCCVC) (SERNAGEOMIN 2015a). This volcanic complex is situated within the limits of the Puyehue National Park, a protected area that includes part of the Andes and pristine temperate evergreen rainforests in southern Chile (ca. 40°5' S, 72°2' W). The PCCVC is composed of four different volcanoes that are aligned in a northwest-southeast axis, oblique to the main volcanic front of the Southern Volcanic Zone of the Chilean Andes (Lara et al. 2006). After decades of quiescence, the PCCVC erupted on June 2011 from a new vent located 7 km from the Puyehue stratovolcano. The initial explosive phase of the eruption spanned for more than two weeks and was characterized by eruption columns that oscillated between 7 and 15 km of altitude (Collini et al. 2013 and references therein). The eruption caused abundant ash fallout not only in the surroundings but

also all around the southern hemisphere (Collini et al. 2013). Currently, the volcano shows a state of quiescence, but wind, rainfall and snowmelt continuously move tephra deposits into nearby basins (SERNAGEOMIN 2015b).

Sampling

In January 2014, we randomly sampled a wide array of microhabitats including litter, decaying wood, topsoil layer and soil mosses in the Puyehue National Park. These microhabitats were selected because they exhibit high levels of testate amoeba species richness in the region (Fernández et al. 2015) and thus their exploration frequently allows having a good representation of the group. A total of 14 samples were collected at different altitudes and distances from the active vent of the PCCVC (Table 1 and Fig. 1). All samples come from sites of the park that were severely impacted by the ash cloud fallout. Notably, samples collected always contained tephra, even when they were collected in very lush and protected sites of the forest. One of the samples collected, unusually rich in volcanic ash (hereafter, the tephra sample), was selected to analyze and characterize the mineral composition of tephra (Table 1).

Sample treatment and ESEM-EDS photographs

Except for the tephra sample, all samples were washed with distilled water and wet sieved through 315 µm mesh sieve to remove debris and other large particles. The lower fraction was examined under a stereomicroscope Olympus SZX16 at 115× magnification to isolate and pick xenosomic testate amoeba species using a very fine paintbrush. A total of 51 amoebae (i.e. 17 species, each represented by three individuals used as statistical replicates), were extracted and positioned on SEM stubs using double-sided adhesive carbon discs and then coated with carbon. The identification of the species was based on morphological features of the

Table 1. Information associated to the microhabitats sampled in the Puyehue National Park, Chile.

Sample code	Coordinates	Altitude (m a.s.l)	Sample type
CHI-27	40°39'48.1" S; 072°10'31.2" W	359	Tephra
CHI-28	40°39'48.1" S; 072°10'31.2" W	359	Soil mosses
CHI-31	40°39'55.6" S; 072°10'22.6" W	340	Litter & topsoil
CHI-36	40°39'38.9" S; 072°10'6.3" W	350	Litter & topsoil
CHI-37	40°39'35.2" S; 072°9'58.3" W	369	Soil mosses
CHI-38	40°39'52.0" S; 072°9'48.0" W	379	Decaying wood
CHI-40	40°39'42.7" S; 072°9'52.7" W	424	Litter & topsoil
CHI-41	40°40'18.9" S; 072°9'58.8" W	410	Litter
CHI-42	40°40'44.0" S; 072°9'47.1" W	441	Soil mosses
CHI-45	40°40'15.9" S; 072°10'9.4" W	388	Decaying wood
CHI-46	40°40'39.0" S; 072°10'10.4" W	566	Soil mosses
CHI-49	40°46'40.9" S; 072°15'31.9" W	787	Litter & topsoil
CHI-52	40°46'3.1" S; 072°17'34.0" W	724	<i>Sphagnum magellanicum</i>
CHI-54	40°46'3.1" S; 072°17'34.0" W	724	Soil mosses



Fig. 1. (a) Geographical localization of the Puyehue-Cordon Caulle volcanic complex (PCCVC) in Chile. (b) Sampling sites in the Puyehue National Park.

shell following the same criteria used by previous taxonomic studies on Chilean testate amoebae (e.g. Fernández and Zapata 2011; Fernández et al. 2012, 2015; Zapata 2005; Zapata and Fernández 2008; Zapata et al. 2008), thus facilitating the comparison of our findings with those of past and future studies in this region.

A fraction of the tephra sample was also put on a stub following the procedure described above for the other samples. Amoeba specimens and tephra on stubs were imaged with an Environmental Scanning Electron Microscope (ESEM) FEI Quanta 200 equipped with an Energy Dispersive X-ray Spectrometer (EDS). Both devices are collectively referred to as ESEM-EDS hereafter. Pictures were taken using both secondary (low-energy) electrons (SEC) and back-scattered (high-energy) electrons (BSE) modes. SEC is the most common imaging mode and facilitates the documentation and morphological identification of the species, while BSE-imaging mode is especially suited to localize areas with high heavy elements content (high Z) because of their stronger reflectivity in their shell and tephra (e.g. areas rich in Fe-Ti-Mg oxides). BSE-imaging also allows getting sharper and crisper images (Erlandsen et al. 1999), facilitating the analysis of the size and morphology of the mineral particles constituting amoeba shells and tephra. BSE images were also used to create a plate with 3D anaglyph images of each amoeba species.

Determining the mineral composition of particles composing tephra and testate amoeba shells

We used ESEM-EDS to conduct both qualitative and quantitative elemental analyzes to determine the structural formula

and then the identity of mineral particles composing the tephra and testate amoeba shells. The qualitative elemental analysis consisted in constructing an elemental map showing the spatial distribution of chemical elements within the tephra and testate amoeba shells. We aimed to map the following 12 chemical elements within each elemental map: Al, Ca, Cl, Fe, K, Mg, Mn, Na, P, S, Si, and Ti. Elemental maps were produced by progressively rastering the electron beam point by point over the visible surface of the tephra and shells. A color code was used to display simultaneously each chemical element on a composite elemental map.

The quantitative elemental analysis consisted of a point elemental analysis. Rather than rastering over a large surface (e.g. as when constructing the elemental map), a point elemental analysis focuses on small areas of interest within the sample down to a few microns, allowing an accurate determination of their chemical compositions. Here, we used elemental maps as guides for locating mineral particles of different colors and thus with potentially different mineral identities. A total of 161- and 23-point elemental analyzes were conducted on specific mineral particles composing tephra and shells, respectively, down to an interactive volume of $1\text{-}\mu\text{m}^3$.

Determining the total mineral composition of tephra and testate amoeba shells

We conducted a stepwise process to estimate the total mineral composition of tephra and each testate amoeba shell collected. The total mineral composition estimation was based on the visible face of the shell impacted by the electron beam. First, we cropped all the mineral particles with the same mineral identity (i.e. those having the same color)

Table 2. Mineral composition of tephra and testate amoeba shells. Values are in percentages. The values were estimated through point elemental analyzes conducted on different mineral particles composing tephra and testate amoeba shells. Elemental analyzes were conducted with an Environmental Scanning Electron Microscope equipped with an Energy Dispersive X-ray Spectrometer. For tephra, total mineral composition does not sum 100% because the percentage of diatom shells was not included (6.34% of the total area of the sample).

Sample/ Replicas number	Data/Statistics	Volcanic glass (%)	Feldspars (%)	Oxides (%)	Quartz (xeno- somes)/amorphous silica (idiosomes) (%)	Total (%)	Analyzed area (μm^2)
Tephra ($n = 1$)		80.40	8.26	4.72	0.28	93.66	42,090.47
<i>Argynnia</i> <i>dentistoma</i> ($n = 3$)	Average SD	27.51 0.54	9.53 0.49	0.12 0.00	62.84 0.23	100	8684.92 2269.85
<i>Centropyxis</i> <i>constricta</i> ($n = 3$)	Average SD	80.81 2.05	11.23 3.75	4.82 0.74	3.14 2.10	100	6742.06 146.54
<i>Centropyxis</i> <i>ecornis</i> ($n = 3$)	Average SD	98.96 0.88	0.97 0.79	0.04 0.06	0.03 0.04	100	27,941.64 796.93
<i>Centropyxis</i> <i>elongata</i> ($n = 3$)	Average SD	95.20 1.82	4.41 1.65	0.38 0.19	0.01 0.01	100	6570.17 1117.37
<i>Centropyxis</i> <i>orbicularis</i> ($n = 3$)	Average SD	95.82 1.04	2.64 0.62	1.17 0.29	0.37 0.31	100	8817.90 2895.33
<i>Centropyxis</i> <i>sylvatica</i> ($n = 3$)	Average SD	90.75 7.61	5.61 3.66	3.09 4.01	0.55 0.78	100	2430.32 289.00
<i>Cyclopyxis</i> <i>eurystoma</i> ($n = 3$)	Average SD	88.60 1.56	4.83 1.68	2.77 0.62	3.79 0.77	100	1002.01 149.67
<i>Diffugia</i> cf. <i>bryophila</i> ($n = 3$)	Average SD	97.47 0.70	1.54 0.07	0.84 0.62	0.15 0.22	100	10,693.97 3690.41
<i>Diffugia</i> cf. <i>lacustris</i> ($n = 3$)	Average SD	96.05 2.49	3.82 2.54	0.13 0.18	0.00 0.00	100	11,243.68 3556.90
<i>Diffugia</i> cf. <i>lata</i> ($n = 3$)	Average SD	97.45 1.54	2.55 1.54	0.00 0.00	0.00 0.00	100	6624.65 2312.80
<i>Diffugia</i> <i>cyphodera</i> ($n = 3$)	Average SD	98.39 0.92	1.21 1.01	0.40 0.12	0.00 0.00	100	21,184.58 8035.55
<i>Diffugia</i> <i>oblonga</i> ($n = 3$)	Average SD	98.34 1.16	1.66 1.16	0.00 0.00	0.00 0.00	100	15,516.29 5207.87
<i>Diffugia</i> sp. ($n = 3$)	Average SD	81.17 2.17	9.73 1.08	5.22 1.66	3.89 0.68	100	1917.14 120.37
<i>Heleopera</i> <i>sylvatica</i> ($n = 3$)	Average SD	98.70 6.14	1.14 4.72	0.00 0.10	0.16 8.67	100	13,350.88 1773.27
<i>Padaungiella</i> sp. ($n = 3$)	Average SD	7.15 4.52	4.16 2.60	0.14 0.20	88.54 6.71	100	8666.93 149.67
<i>Plagiopyxis</i> <i>declivis</i> ($n = 3$)	Average SD	99.10 0.93	0.73 1.03	0.17 0.24	0.00 0.00	100	4883.98 464.67
<i>Schwabia</i> <i>regularis</i> ($n = 3$)	Average SD	85.12 3.10	5.72 1.09	5.31 0.69	3.85 3.17	100	4855.37 511.86

from elemental maps representing the spatial distribution of mineral particles within tephra and testate amoeba shells. The mineral particles with the same mineral identity were then grouped and their total area calculated using the software ImageJ 1.05b. This calculation allowed us to know the area occupied by a given mineral in a given elemental map (Supplementary material 1). Determining the total

area occupied by minerals in the elemental maps allowed us to estimate the total mineral composition of tephra and of each testate amoeba shell. Finally, we used the total mineral composition values obtained for each one of the three shells belonging to the same species (i.e. replicas) to calculate the arithmetic mean (average) of the total mineral composition of the respective taxon (Table 2).

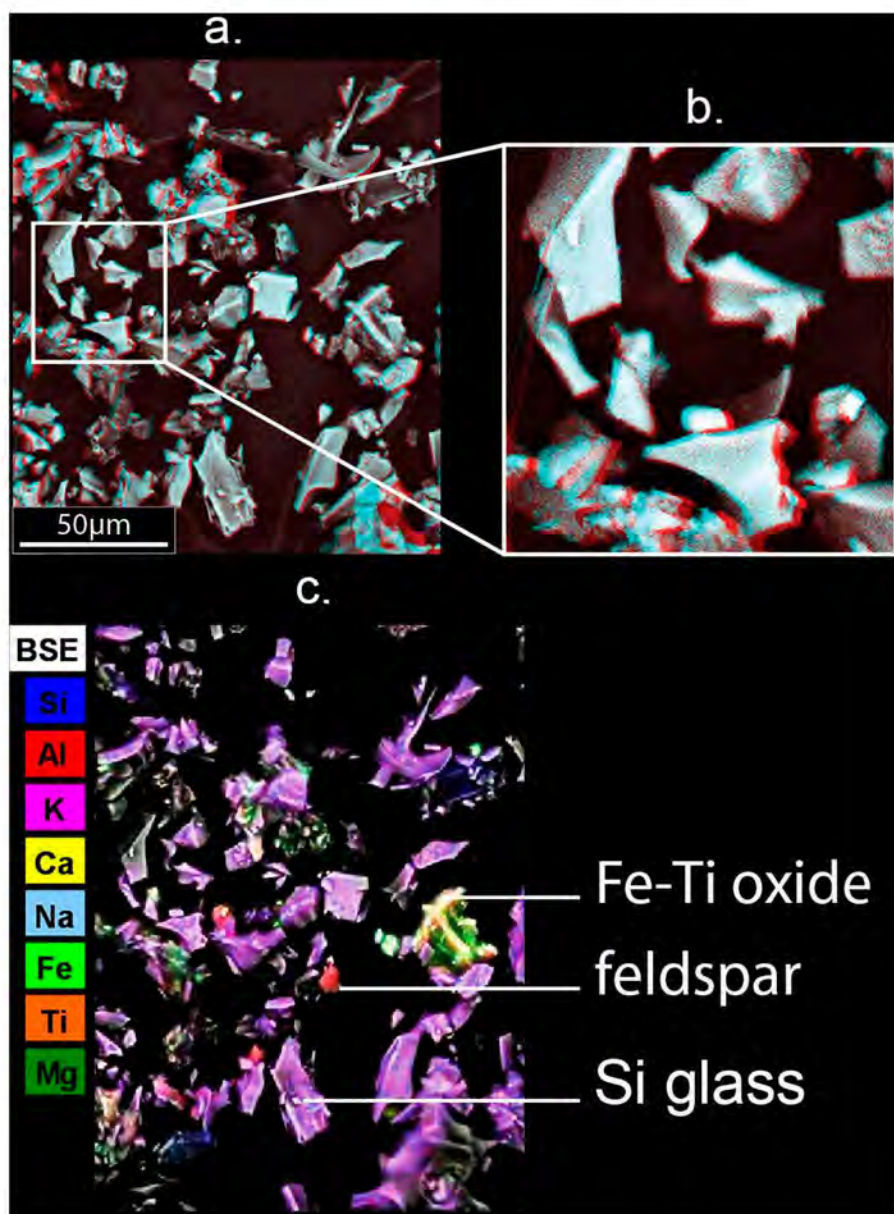


Fig. 2. (a) 3D red/cyan anaglyph micrograph showing the morphological characteristics of tephra particles erupted on 2011 by the Puyehue-Cordón Caulle volcanic complex, Chile. (b) Zoom in of tephra particles showing sharp edges and conchoidal fractures. (c) Composite elemental map showing the chemical elements present within tephra. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ejop.2016.05.003>.

Results and Discussion

Mineral composition of particles composing tephra

Observations under binocular and BSE-imaging showed that tephra was essentially vitreous, mainly composed of

white to dark gray glassy particles with sizes ranging from 1 to 350 µm. These particles exhibited sharp edges and conchoidal fractures (Fig. 2a,b), suggesting that tephra from the PCCVC was mainly composed of silica-rich volcanic glass (Dürig and Zimanowski 2012). This was further confirmed by both the composite elemental map and the set of 23-point elemental analyzes (Fig. 2c, Table 2, and Supplementary material 2). The point elemental analyzes conducted only on transversely cut volcanic glass particles composing the tephra sample confirmed its chemical heterogeneity (Supplementary material 3). These point elemental analyzes also revealed that volcanic glass particles composing the tephra sample mostly

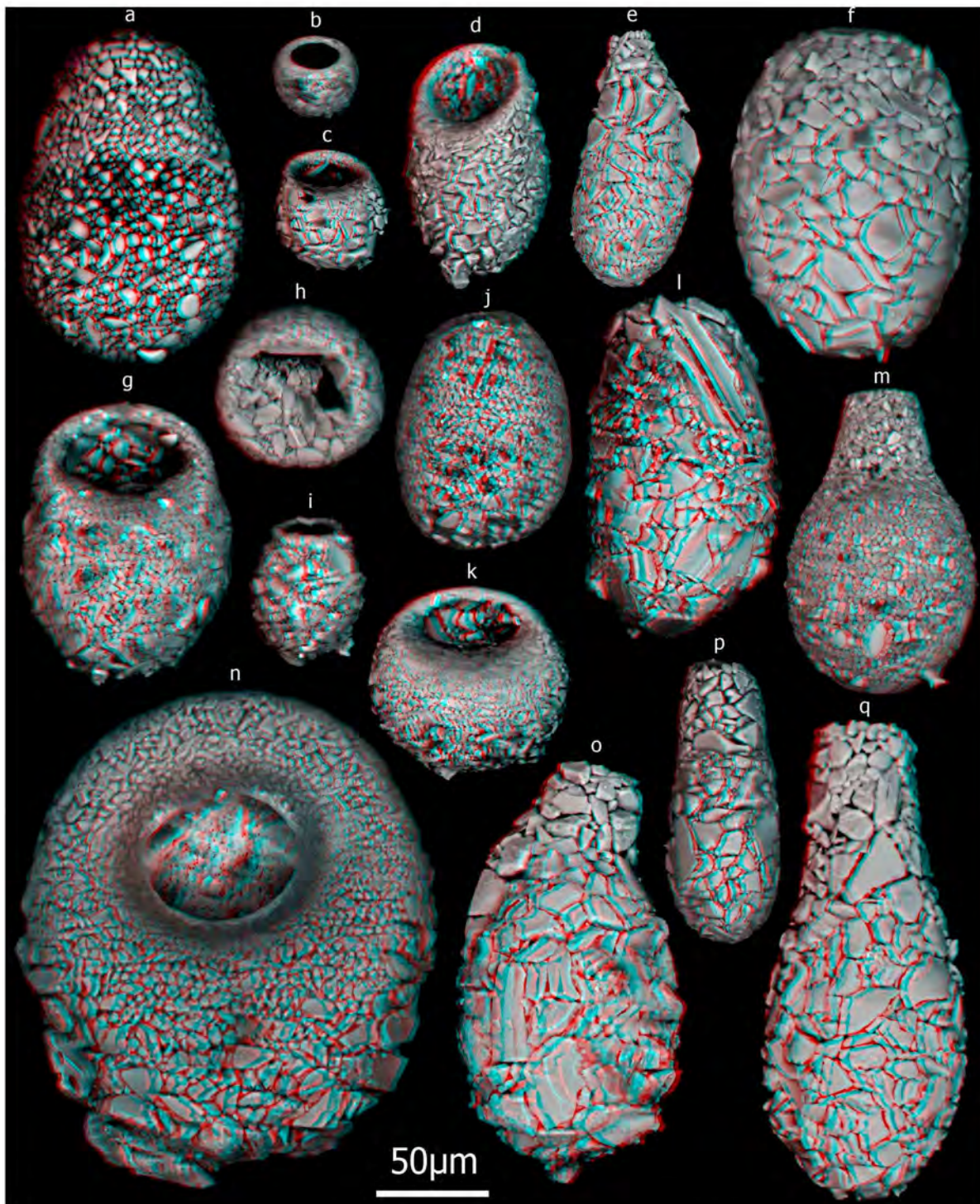


Fig. 3. 3D anaglyph micrographs of testate amoeba species recorded in the Puyehue National Park, Chile. (a) *Argynnia dentistoma* (Penard, 1890), (b) *Cyclopyxis eurystoma* Deflandre, 1929, (c) *Centropyxis sylvatica* (Deflandre, 1929), (d) *Centropyxis elongata* (Penard, 1890), (e) *Diffugia* cf. *bryophila* (Penard, 1902), (f) *Heleopera sylvatica* Penard, 1890, (g) *Centropyxis constricta* (Ehrenberg, 1841), (h) *Plagiopyxis declivis* Bonnet and Thomas, 1955, (i) *Diffugia* sp., (j) *Schwabia regularis* Jung, 1942, (k) *Centropyxis orbicularis* Deflandre, 1929, (l) *Diffugia* cf. *lata* (Jung, 1942), (m) *Padaungiella* sp. (undescribed species, Fernández et al. unpublished data), (n) *Centropyxis ecornis* (Ehrenberg, 1841), (o) *Diffugia cyphodera* (Jung, 1942), (p) *Diffugia* cf. *lacustris* (Penard, 1899), (q) *Diffugia oblonga* Ehrenberg, 1838.

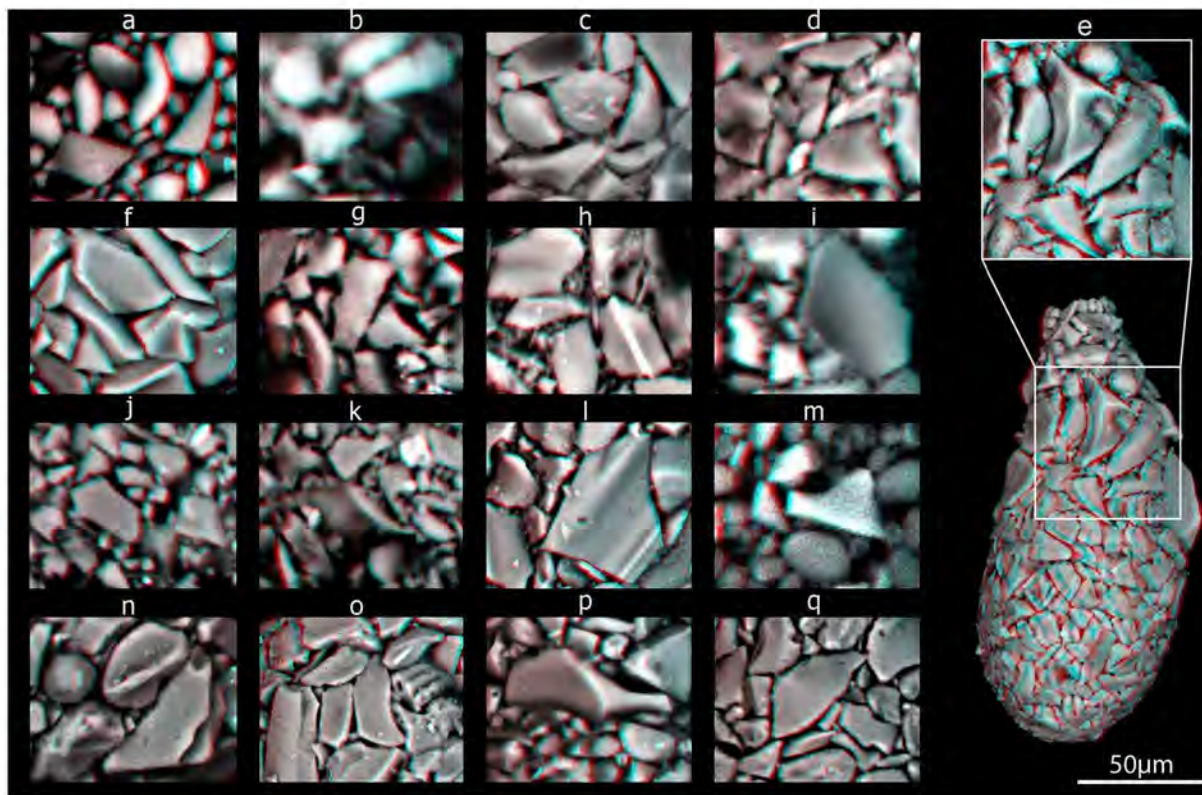


Fig. 4. Detail of mineral particles and xenosomes composing testate amoeba shells. Letters correspond to species as in Fig. 3.

were of dacitic and rhyolitic types (Supplementary material 4). These volcanic glass types are classified as acidic igneous rocks (Le Maitre et al. 2002). Our quantitative and qualitative results were closely consistent with previous studies on tephra from the PCCVC (e.g. Buteler et al. 2011; Caneiro et al. 2011; Collini et al. 2013).

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Mineral composition of particles composing testate amoeba shells

Seventeen species were isolated from the samples (Fig. 3). BSE-imaging showed that mineral particles composing testate amoeba shells exhibited the same morphological characteristics quoted above for tephra from the PCCVC (i.e. mineral particles with sharp edges and conchoidal fractures), suggesting that they were of volcanic origin (Fig. 4). Both qualitative and quantitative elemental analyzes supported this idea. First, the elemental maps revealed that chemical elements have similar proportions to those found in the tephra (Fig. 5 and Supplementary material 2). As in the tephra, the most abundant chemical element on testate amoeba shells was silica, composing about 39–72% of the total analyzed area (Supplementary material 2). Next, the set of 126-point elemental analyzes conducted on different mineral particles

composing testate amoeba shells revealed that 15 of the 17 species identified had a shell mostly built with silica-rich volcanic glass particles, while the shell of the other two species (i.e. *Argynnia dentistoma* (Penard, 1890) and *Padaungiella* sp.) were mainly built with idiosomes recycled from other species (Fig. 6, Table 2 and Supplementary material 5). This finding was not surprising, since the species of the genera *Argynnia* Vucetich, 1974 and *Padaungiella* Lara et Todorov, 2012 commonly use mineral particles only as a complement to build their shell (e.g. Clarke 2003; Mazei and Tsyganov 2006; Ogden and Hedley 1980; Zapata et al. 2007). Our results also revealed that *Heleopera sylvatica* Penard, 1890 had a shell almost completely built with volcanic glass particles (98.7% of the surface of its shell was coated with this material) (Fig. 6, Table 2). While it is known that in this species the proportion of mineral particles used to build the shell can vary from among individuals (e.g. Clarke 2003), to our knowledge this is the first report for this species of a shell almost totally built with mineral particles (i.e. volcanic glass). This suggests that this species does not have a preference for any building material and that, like other testate amoeba species (Štěpánek, 1952; Armynot du Châtelet et al. 2010, 2015), it simply uses the most abundant building material in its habitat to build its shell (i.e. tephra in our study). Moreover, it may suggest that *H. sylvatica* prefers mineral particles as building material when they are in sufficient abundance and appropriate size in its habitat. These interpretations should however be confirmed by further

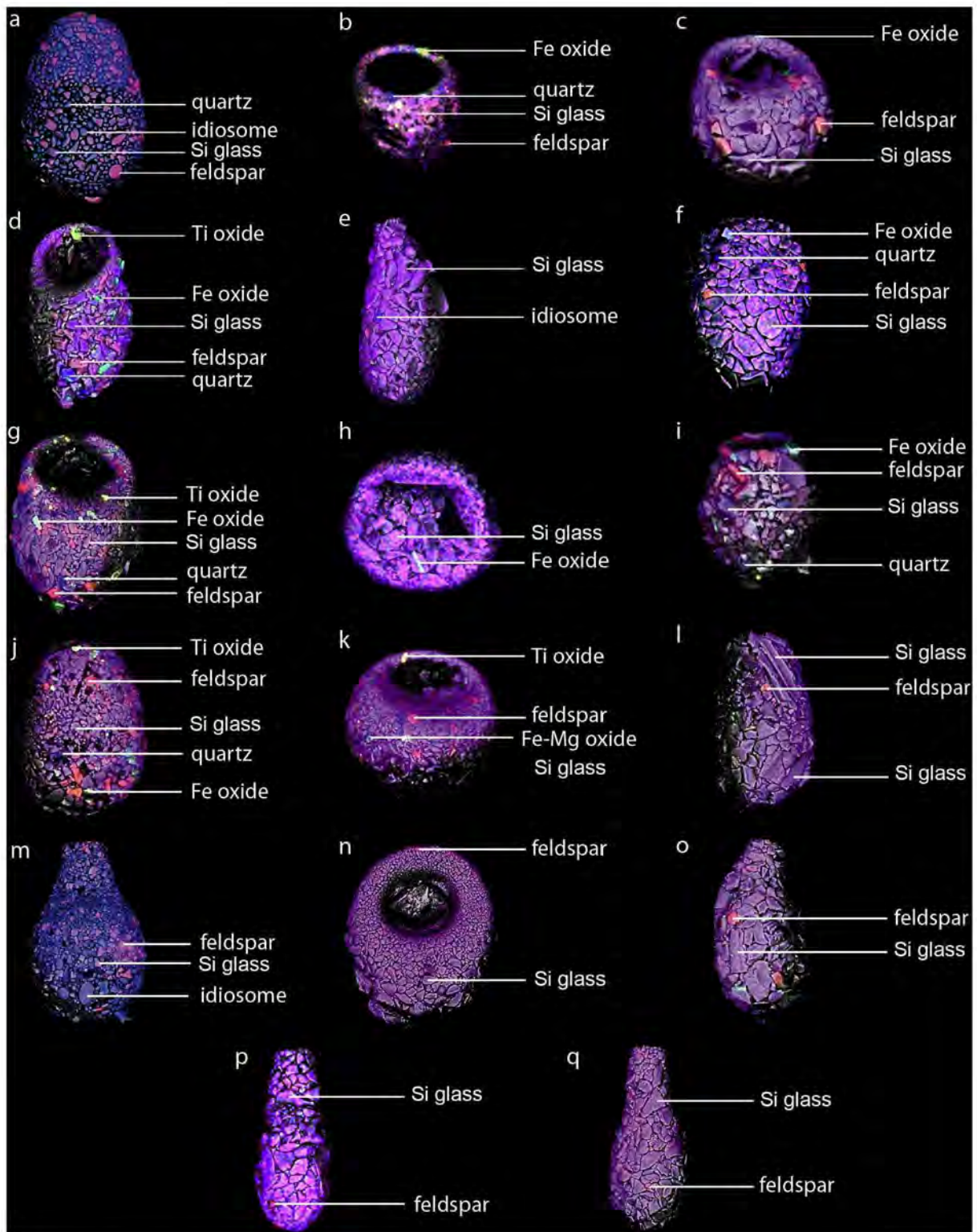
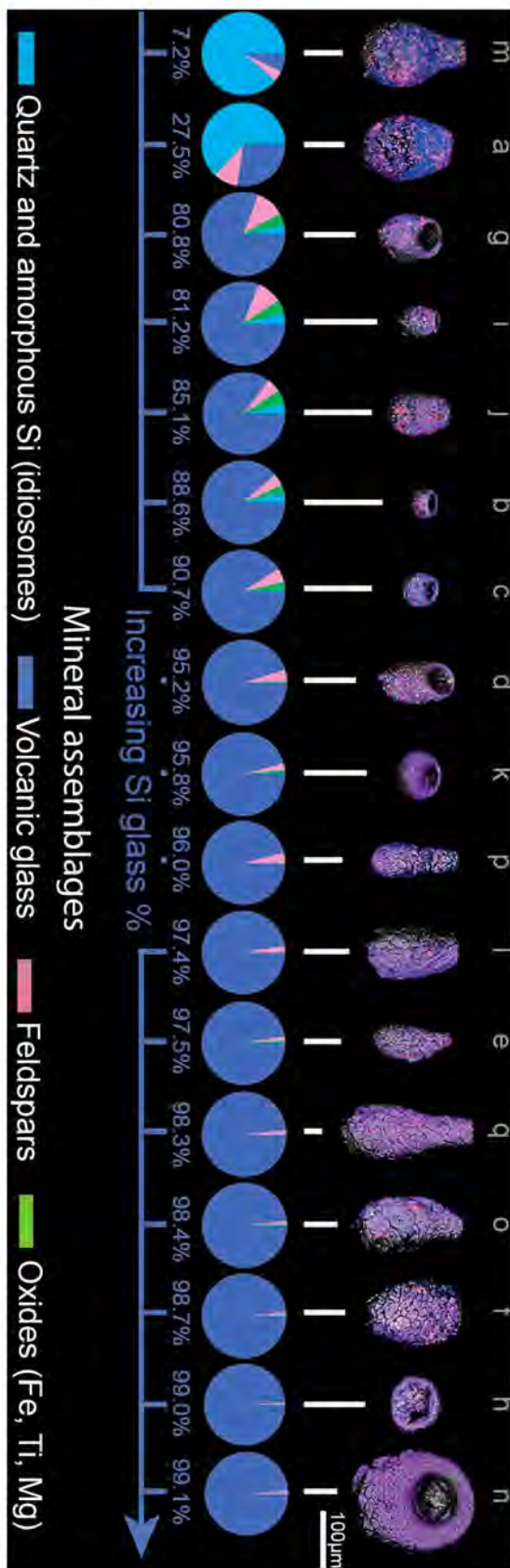


Fig. 5. Composite elemental maps showing the identity of the mineral particles composing testate amoeba shells. Letters correspond to species as in Fig. 3.

studies and especially taking into account the possible existence of several taxa within this morphospecies, which may differ in the use of xenosomes for building their shell. This observation could also apply to other species that, according

to the literature, barely use mineral particles as building material (e.g. *Argynnia* spp. and *Padaungiella* spp.). However, we can only speculate on this question, and further research is needed to address it properly.



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Additional point elemental analyzes conducted on very thin and polished sections of volcanic glass particles composing testate amoeba shells revealed that they mostly were of trachytic type and, to a lesser extent, of dacitic and rhyolitic types, (Supplementary material 4). While this analysis confirmed that testate amoebae used tephra to build their shells, it also revealed that the volcanic glass type that dominated in testate amoeba shells was different from that prevailing in the tephra (i.e. dacitic and rhyolitic types).

As volcanic glass particles of dacitic and rhyolitic types are much more acidic than those of trachytic type (Le Maitre et al. 2002), one can hypothesize that testate amoebae select the much more alkaline trachytic particles from tephra, possibly because it improves the performance of the organic cement that they use to bind together the xenosomes. This interpretation would agree with evidence showing that the organic cement produced and used by some other shelled eukaryotic microorganisms (e.g. foraminifera) to construct their agglutinated shells does not perform well under acidic conditions (Scott et al. 2001; Fernández and Zapata 2010a, 2010b).

However, it should be noted that the study site has also experienced the ash fallout of other past eruptions produced by the PCCVC and other neighboring volcanoes (Buteler et al. 2011; Caneiro et al. 2011; Collini et al. 2013). Therefore, it is also possible that the chemical composition of the volcanic glass found in testate amoeba shells mirrors the diversity of volcanic glass found in the soil of the study site, which results from past eruptions as well as the current eruption of the PCCVC. This would imply that testate amoebae are not selecting any particular volcanic glass type to build their shells. Rather, they would be randomly selecting any volcanic glass particle that it is in abundance in their immediate environment (Armynot du Châtelet et al., 2010).

Alternatively, the collected samples may also have included some subfossil shells built with volcanic glass particles from past eruptions. However, we consider this possible bias as unlikely as samples were taken from the topsoil (litter and upper organo-mineral) horizon or in microenvironments lying at the topsoil and all species studied were observed alive in the samples. Whatever the reasons of the aforementioned difference between the volcanic glass type dominating in tephra and testate amoeba shells, one fact is certain; our quantitative and qualitative elemental analyzes demonstrated that mineral particles present in testate amoeba shells were of volcanic origin, thus supporting our hypothesis. To the

Fig. 6. Total mineral composition of the shell of testate amoeba species collected in the Puyehue National Park, Chile. The chart mainly focuses on showing the amount (%) of volcanic glass particles agglutinated by each species to build its shell. See Fig. 3 for species identity and Table 2 for the identity and amount of other mineral particles used by the species to build their shells.

best of our knowledge, there are no other studies reporting that testate amoebae use tephra to build their shells in a site impacted by a volcanic eruption.

Conclusions

We have demonstrated that testate amoebae can act as Cinderella's helping pigeons of the microbial world, since they collect and sort tephra particles to build their shells in sites affected by the ash fallout from volcanic eruptions. By building a shell 10–100 times larger than most of the non-visible volcanic ash particles, they can facilitate the extraction and counting of cryptotephra from the host sediment, helping researchers to save time on tasks that otherwise would be tiresome and laborious. By contrast, the isolation of testate amoeba shells (some of which are large enough to be isolated with a dissecting microscope) is less-time consuming. By picking individual testate amoeba shells of a given species from different layers of a sediment or peat core (or from different soil samples) and analyzing their mineral composition using X-ray spectrometry, it should be possible to determine the presence or absence of cryptotephra more easily and possibly more precisely than the analysis of the matrix itself.

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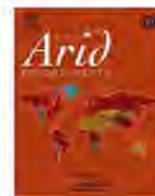
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Appendix D. Source-sink dynamics shapes the spatial distribution of soil protists in an arid shrubland of northern Chile



Source–sink dynamics shapes the spatial distribution of soil protists in an arid shrubland of northern Chile



Leonardo D. Fernández ^{a, b, c, *}

^a Laboratory of Soil Biology, Institute of Biology, University of Neuchâtel, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland

^b Laboratorio de Ecología Evolutiva y Filoinformática, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario s/n, Casilla 160-C, Concepción, Chile

^c Centro de Estudios en Biodiversidad (CEBCH), Magallanes, 1979 Osorno, Chile

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ABSTRACT

Arid shrublands are spatially heterogeneous environments composed of shrubs patches surrounded by a matrix of bare soil. In this biome, bare soils harbor fewer protist species than soils covered by shrubs because they represent low quality habitats for most of them. Furthermore, protist species composition in bare soils often represents a subset of that found in soils covered by shrubs. This pattern suggests that the variability in protist species composition is the result of a non-random mechanism. I performed a nested analysis to test the hypothesis that the source–sink dynamics is shaping the spatial distribution of soil testate amoebae (a polyphyletic group of protists) in an arid shrubland of northern Chile. I found that assemblages of soil testate amoebae are significantly nested in this biome. The species-poor assemblages inhabiting bare soils are thus, only a subset of species-rich assemblages located in vegetated soils. This nested pattern may be the result of a colonization–extinction tradeoff characterized by species-poor bare soils acting as ‘sinks’ maintained by the permanent colonization of individuals coming from species-rich vegetated soils acting as ‘sources’. Thus, the source–sink dynamics represents a valid conceptual framework to explain the spatial patterns of soil protists distribution in arid shrublands.

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1. Introduction

Arid shrublands are fragmented landscapes composed of large areas of bare soil and widely separated shrub patches. In this biome, bare soils harbor fewer protist species than soils covered by shrubs because they represent low quality habitats for most of them, e.g. low water and nutrient content, high desiccation risk (Bamforth, 2008; Robinson et al., 2002). Instead, soils located under shrubs comprise higher quality habitats for protists, since plants provide shading, create heterogeneity in soils, intercept and retain water, and concentrate nutrients under their canopy (del-Val et al., 2006; Schlesinger et al., 1996). In addition, studies of protist species composition in arid shrublands often found no species replacement (i.e. species turnover) between bare soils and those covered by shrubs (Bamforth, 2008; Robinson et al., 2002). Variation in species composition between both habitats is thus only due to differences in species richness and protist species

composition of bare soils only represents a subset of that found in soils beneath shrubs. This suggests that the variability in protist species composition is the result of a non-random mechanism such as source–sink dynamics. This model predicts that species-poor assemblages inhabiting sink (low quality) habitats are constituted by non-viable populations maintained by a constant colonization of individuals coming from species-rich assemblages located in source (high quality) habitats (Pulliam, 1988). As a result a nested subset pattern of species composition occurs, in which impoverished sites tend to be proper subsets of the richer sites (Atmar and Patterson, 1993; Wright et al., 1998). The empirical study of the source–sink dynamics is a difficult task because it requires long-term studies that often are impracticable. However, the ecological concept of nestedness provides an alternative and robust framework to indirectly infer the relative importance of the source–sink dynamics in shaping the spatial distribution of species. Nestedness predicts that when depauperate sites tend to be simple subsets of richer ones, a nested subset pattern of species composition emerges, suggesting a highly ordered system in which both colonization and extinction may be actively shaping species occurrence across sites (Atmar and Patterson, 1993; Wright et al., 1998). In arid shrublands, nested assemblages of soil protists

* Corresponding author. Laboratory of Soil Biology, Institute of Biology, University of Neuchâtel, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland.

E-mail addresses: limnoleo@gmail.com, leonardo.fernandez@unine.ch.

may imply that the spatial variation in species composition is determined by source–sink dynamics, where sink (bare soils) habitats would be characterized by high extinction rates, so species richness in those zones should be maintained mainly by the permanent colonization of individuals coming from source (vegetated soils) habitats, where populations would be actively growing and reproducing.

In this study, I tested the hypothesis that the source–sink dynamics is shaping the spatial patterns of soil protists distribution in arid shrublands. For this task I searched for the occurrence of a nested subset pattern of species composition among assemblages of testate amoebae inhabiting an arid shrubland of northern Chile. Testate amoebae are a polyphyletic group of protists (composed mainly by Cercozoa and Arcellinida) in which the amoeboid body is protected by a shell. The shells show a wide range of morphological adaptations related to the availability of soil water. Overall, dry and bare surface soils favor species with cryptostome or plagiostome shell types (i.e. shells with a well-protected or hidden aperture). Meanwhile, stable soils with medium–high moisture availability allow the occurrence of species with axial and acrostome shell types (i.e. terminal or well visible aperture). Testate amoebae inhabiting arid soils of northern Chile have not been documented before, so this study also aimed to expand our knowledge on eukaryotic microorganisms that inhabit this region.

2. Materials and methods

2.1. Study site

In March 2007, I studied a 10-km² area of an arid shrubland in the Coquimbo Region of northern Chile. The study site was located on the southern edge of the Atacama Desert about 40 km from the Pacific Coast (29°59' S; 70°47' W). The regional climate is characterized as mediterranean-arid with hot dry summers and cool winters (del-Val et al., 2006). Mean annual temperature is 13.6 °C and mean annual precipitation is 147 mm, nearly 95% of which falls during the winter (del-Val et al., 2006). The vegetation of the study site is shrub–steppe type and consists of vegetation patches surrounded by a matrix of bare soil (Gajardo, 1994).

2.2. Sampling and sample treatment

I randomly located ten 100-m long transects in the study site and extracted 10-cm³ of soil at each 20-m interval. For each sample I noted whether it was taken from beneath vegetation or from bare soil. Within 1–2 days, samples were washed through 500 and 300 µm mesh sieves to remove the coarse material. Each sample was explored by using direct observation and by culture methods for documenting species occurrence. The use of cultures allowed for the detection of species that were encysted¹ at the time of sampling. Cultures were prepared following the procedures described by Wilkinson and Smith (2006) and examined for the presence of organisms after 2, 4, 6, 8, 10, 20, 40 and 60 days of incubation at 20 °C. The identification and further sorting of taxa in different shell types were based on traditional references (Bonnet, 1975; Fernández et al., 2012 and taxonomic references cited therein). Species occurrence data were used to construct a binary matrix (i.e. columns as samples and rows as taxa) on which all subsequent statistical analyses were based.

¹ A cyst is a dormant stage that allows the organism to survive under adverse environmental conditions, e.g. during droughts. The encysted organisms may be overlooked by direct observation methods.

2.3. Statistical analyses

Mann–Whitney *U*-tests were performed in PAST v1.5 (Hammer et al., 2001) to test whether bare and vegetated soils differ in: (1) testate amoeba species richness; and (2) the number of occurrences of different shell types (cryptostome–plagiostome versus axial–acrostome). Statistical inference was based on a random permutation test (10,000 iterations) to calculate the 95% confidence intervals.

To rule out possible bias in the sampling effort, a species–accumulation curve based on the Chao2 estimator was calculated in EstimateS v9.1 (Colwell, 2006). This method estimates what proportion of the total diversity of a site has been detected by plotting the observed number of species as a function of the accumulated number of samples. Statistical inference was based on a random permutation test (50,000 iterations) to calculate the 95% confidence intervals.

The NODF index was used to explore the occurrence of a nested subset pattern of species composition in the matrix. This index was also calculated independently for columns and rows of the matrix. Nestedness among columns represents differences in species richness and nestedness among rows represent differences in species incidence. Decomposing nestedness into its among-column and among-row components allow calculating to which extent gradients in the characteristics of sites (habitat

Table 1

Testate amoeba species recorded in a 10-km² area of an arid shrubland of northern Chile.

Species	Number of occurrences				
	Study site	Under plants	Bare soil	Shell type	Note
<i>Arcella arenaria</i> Greeff, 1866	37	25	12	AX	a
<i>Arcella vulgaris</i> Ehrenberg, 1830	24	18	6	AX	c
<i>Argynnia</i> sp.	6	3	3	AC	a
<i>Centropyxis aculeata</i> (Ehrenberg, 1838)	35	17	18	PL	c
<i>Centropyxis aerophila</i> Deflandre, 1929	28	17	11	PL	a
<i>Centropyxis constricta</i> (Ehrenberg, 1841)	19	11	8	PL	a
<i>Centropyxis discoides</i> (Penard, 1890)	21	12	9	PL	a
<i>Centropyxis plagiostoma</i> Bonnet and Thomas, 1955	42	25	17	PL	a
<i>Cryptodiffugia oviformis</i> Penard, 1890	13	9	4	AC	a
<i>Cyclopyxis eurytoma</i> Deflandre, 1929	31	16	15	AX	a
<i>Cyclopyxis humilis</i> Bonnet, 1962	13	6	7	AX	a
<i>Cyclopyxis kahli</i> (Deflandre, 1929)	15	8	7	AX	a
<i>Cyclopyxis</i> sp.	9	5	4	AX	a
<i>Diffugia distenda</i> (Penard, 1989)	8	6	2	AC	a
<i>Diffugia oblonga</i> Ehrenberg, 1838	15	10	5	AC	a
<i>Euglypha cristata acicularis</i> Wailes, 1912	14	7	7	AC	a, b, d
<i>Euglypha strigosa</i> (Ehrenberg, 1871)	11	6	5	AC	a
<i>Euglypha tuberculata</i> Dujardin, 1841	7	4	3	AC	a
<i>Netzelia wailesi</i> (Ogden, 1980)	11	9	2	AC	a
<i>Phryganella acropodia</i> (Hertwig and Lesser, 1874)	17	12	5	AX	a
<i>Phryganella</i> sp.	5	3	2	AX	a
<i>Plagiopyxis</i> cf. <i>declivis</i> Bonnet and Thomas, 1955	32	22	10	CR	a
<i>Scutiglypha cabrolae</i> De Smet and Gibson, 2009	21	12	9	AC	c
<i>Scutiglypha crenulata</i> (Wailes, 1912)	21	11	10	AC	a, b
<i>Scutiglypha scutigera</i> (Penard, 1911)	7	7	0	AC	a, b, d
<i>Tracheleuglypha dentata</i> (Penard, 1890)	14	9	5	AC	a
<i>Trinema complanatum</i> Penard, 1890	9	6	3	PL	a
<i>Trinema enchelys</i> (Ehrenberg, 1838)	24	15	9	PL	a, d
<i>Trinema lineare</i> Penard, 1890	29	18	11	PL	a

AC = acrostome shell type; AX = axial shell type; CR = cryptostome shell type; PL = plagiostome shell type; a = first record for northern Chile; b = first record for southwestern South America; c = species previously recorded in northern Chile; d = species only found in cultures. Maximum possible number of occurrences per species = 50.

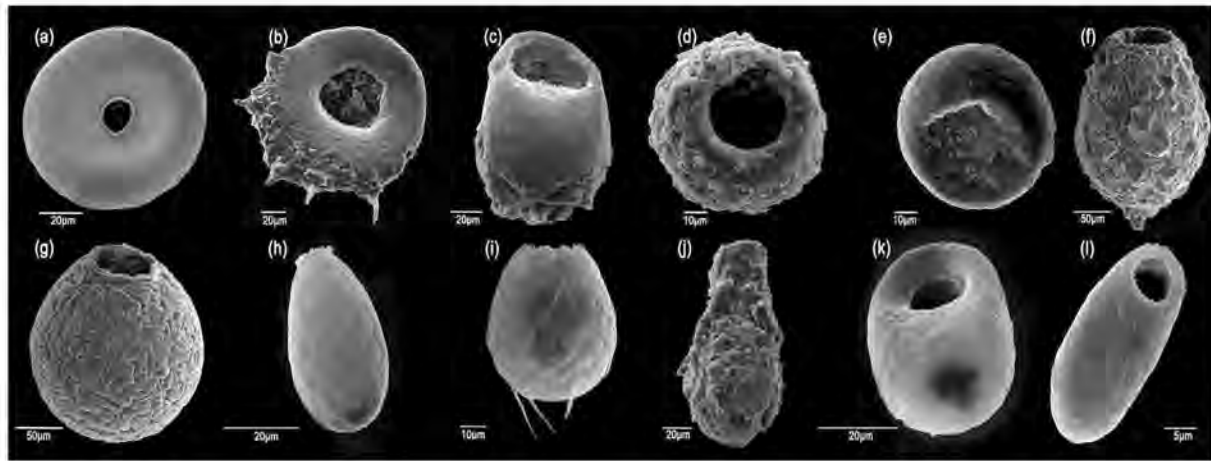


Fig. 1. Scanning Electron Microscope pictures of some testate amoebae exhibiting the morphological shell types observed in the study site. AC = acrostome shell type; AX = axial shell type; CR = cryptostome shell type; PL = plagiostome shell type. (a) *Arcella arenaria* Greeff, 1866 (AX); (b) *Centropyxis aculeata* (Ehrenberg, 1838) (PL); (c) *Centropyxis constricta* (Ehrenberg, 1841) (PL); (d) *Cyclopyxis eurytoma* Deflandre, 1929 (AX); (e) *Plagiopyxis cf. declivis* Bonnet and Thomas, 1955 (CR); (f) *Diffugia distenda* (Penard, 1899) (AC); (g) *Netzelia walesi* (Ogden, 1980) (AC); (h) *Tracheleuglypha dentata* (Penard, 1890) (AC); (i) *Scutiglypha cabrolae* De Smet and Gibson, 2009 (AC); (j) *Diffugia oblonga* Ehrenberg, 1838 (AC); (k) *Trinema complanatum* Penard, 1890 (PL); (l) *Trinema lineare* Penard, 1890 (PL). For scanning electron microscopy, the individuals were cleaned by several transfers through distilled water, mounted on coverslips and finally air-dried. The individuals on coverslips were coated with gold and examined with a JEOL JSM-6380 operating at 15 kV.

quality) and traits of species (shell type) contribute to the whole-matrix nestedness (Almeida-Neto and Ulrich, 2011). The NODF index ranges from 0 to 100, where high values are consistent with higher degrees of nestedness. The statistical significance of these analyses was evaluated with a row–column null-model (50,000 permutations, $p < 0.05$), a conservative algorithm that minimizes Type I errors (Ulrich and Gotelli, 2010). Nestedness and null-model calculations were done using NODF v2.0 (Almeida-Neto and Ulrich, 2011).

3. Results

I observed 29 testate amoeba taxa in the 50 analyzed samples, including three taxa that could not be identified to species level. All but three species are reported here for the first time in northern Chile. Four of these species also represented first records for southwestern South America. Nine species exhibited a cryptostome or plagiostome shell type, while the remaining 20 species showed an axial or acrostome shell type. A detailed species list, including their shell types and occurrences in the habitats are given in Table 1. Fig. 1 shows some testate amoeba species and shell types observed in the study site.

In most cases testate amoeba taxa were found by using a direct observation method, suggesting that they were ecologically active in the study site. Only two additional taxa were found after using a culture method (Table 1). The exploration of the cultures after 20, 40 and 60 days revealed no new taxa on plates, indicating that all the diversity present in the samples was found (Fig. 2A). Furthermore, the species–accumulation curve showed that all data points fell within the 95% confidence intervals estimated with Chao2 (Fig. 2B) and thus that sampling was sufficiently exhaustive to obtain a reasonable estimate of the total diversity in the studied habitats.

Soil samples collected from beneath vegetation exhibited a higher number of testate amoeba species than bare soils (Fig. 3A). Cryptostome and plagiostome shell types were widely distributed, as they exhibited a greater number of occurrences than the species with an axial and acrostome shell types in both vegetated soils and bare soils (Fig. 3B and C).

The NODF index value obtained for the whole matrix revealed that testate amoeba assemblages are significantly nested at the study site (Table 2). The NODF index value for columns and rows confirmed that this nested pattern is due to a simultaneous species overlap from species-poor sites to species-rich sites and co-occurrence from limited to widely distributed species (Table 2).

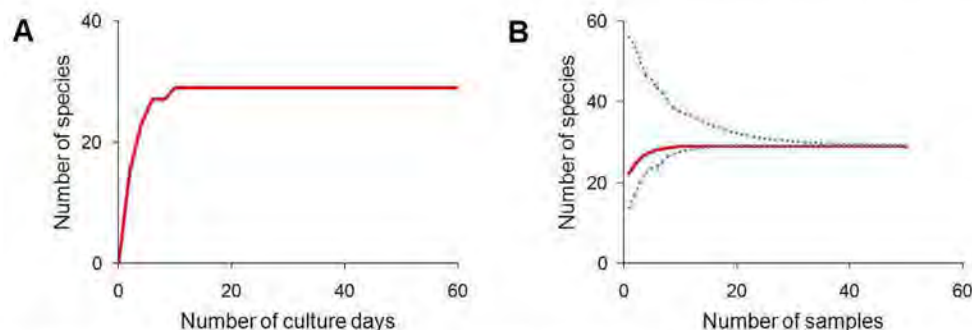


Fig. 2. Number of testate amoeba species: (A) recorded as a function of the cumulative number of culture days. The plot does not consider fluctuations in species abundance, but only the species occurrence in cultures after 60 days of incubation at 20 °C (broken line); and (B) predicted by Chao2 estimator (species–accumulation curve) as a function of the sampling effort (solid line) and the lower and upper 95% confidence intervals of the null distribution of 50,000 random permutations (broken lines) sampled without replacement from the observed dataset (number seed = 123456789).

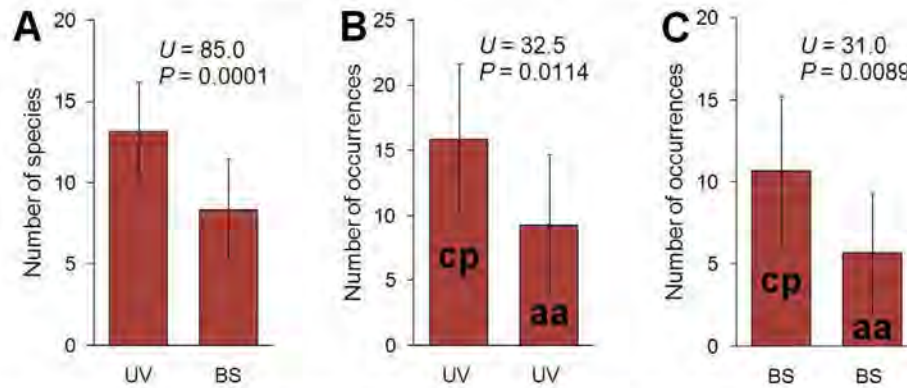


Fig. 3. Mann–Whitney U -tests (U) performed to test whether soils under vegetation (UV) and bare soils (BS) differ in: (A) testate amoeba species richness; and (B, C) the number of occurrences of different shell types (cryptostome–plagiostome type [cp] and axial–acrostome type [aa]). Statistical inference was based on a Monte Carlo permutation test (10,000 iterations) to calculate the 95% confidence intervals. Error bars represent standard deviation.

4. Discussion

This study provided strong support for the hypothesis that the source–sink dynamics is shaping the spatial patterns of soil protist distribution in arid shrublands. Here, testate amoeba species richness was higher in vegetated soils than in bare soils and only those species with cryptostome and plagiostome morphological traits were widely distributed in harsh soils. In line with these results, testate amoeba assemblages were significantly nested at the study site, and nestedness components showed that this pattern was simultaneously caused by connected gradients in the characteristics of sites and traits of species. Nestedness among-columns confirmed that species composition of bare soils was a proper subset of that found in soils covered by shrubs, pointing out that all the variation in species composition was due to differences in species richness between both habitats; i.e. there was no species turnover. Meanwhile, nestedness among-rows demonstrated that less distributed species (axial–acrostome shell types) were found in a subset of the sites in which those species with higher incidence occurred (cryptostome–plagiostome shell types). The observed nested pattern was robust to possible methodological artefacts, since these pitfalls were avoided and controlled by using two independent and complementary methods: cultures and species–accumulation curve. Cultures allowed the detection of all species that were present in the samples, including those that were encysted. The species–accumulation curve confirmed that very few additional species would be recorded with a higher sampling effort. This comprehensive picture strongly suggests that the spatial patterns of soil testate amoebae distribution is the result of a colonization–extinction tradeoff characterized by species–poor bare soils acting as ‘sinks’ maintained by the permanent colonization of individuals coming from species-rich vegetated soils acting as ‘sources’. Thus, the source–sink dynamics represents a valid

conceptual framework to explain the spatial patterns of soil protists distribution in arid shrublands.

A core question to source–sink theory is why individuals would leave a high quality habitat for another of lower quality, where high mortality could result in a population decline toward extinction. Soil protists disperse passively, relying mainly on wind to colonize other sites (Foissner, 1987). As a result, source–sink dynamics can arise simply because external agents dispersed propagules, forcing individuals to grow in a poor habitat (Remes, 2000). However, this does not imply that all soil protists are equally likely to disperse and colonize low quality habitats in arid shrublands. In soil, the distribution (both horizontal and vertical) of protists is mainly determined by the extent to which the species exhibit traits that allow them to avoid dehydration (Bonnet, 1975). Consequently, only a selected subset of taxa occurring in vegetated soils can colonize bare soils (Bamforth, 2008), leading to the observed nested pattern of species composition. In this study, the selected subset of taxa was represented by species with cryptostome and plagiostome morphological traits. These taxa are more abundant in the less stable upper soil horizons (Foissner, 1987) and can establish populations in bare soils because they tolerate better stressful conditions (Bamforth, 2008; Robinson et al., 2002). Accordingly, they are more likely to be passively dispersed than those taxa with axial–acrostome morphological traits and/or living below-ground (Fernández and Zapata, 2011; Mitchell and Meisterfeld, 2005). Thus, while the source–sink dynamics may arise primarily from the passive dispersal of soil protists, the impoverished assemblages of protists inhabiting bare soils may primarily be due to the continuous and differential immigration of species that exhibit both traits to withstand stressful abiotic conditions and a shallow vertical distribution in soil. Finally, these results suggest that the source–sink dynamics also could be shaping the spatial patterns of soil protists distribution in other fragmented landscapes as well as in those exhibiting gradients in habitat quality.

Table 2

Degree to which species-poor assemblages inhabiting bare soils are subsets of species-rich assemblages located in vegetated soils in a 10-km² area of an arid shrubland of northern Chile. Nestedness was based on a presence–absence data matrix ordered according to the marginal row/column sums. Below are shown the observed ($NODF_{obs}$) and expected ($NODF_{exp}$) values of $NODF$ index for the whole matrix ($NODF_{matrix}$), columns ($NODF_{columns}$) and rows ($NODF_{rows}$). Statistical inference was based on the lower and upper confidence limits (L95%CL, U95%CL, respectively) of the null distribution of 50,000 randomized matrices.

	$NODF_{obs}$	$NODF_{exp}$	L95%CL	U95%CL	P-value
$NODF_{matrix}$	45.71	32.49	30.26	34.70	0.002
$NODF_{columns}$	46.12	32.52	30.14	34.85	0.002
$NODF_{rows}$	44.48	32.41	30.30	34.48	0.002

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jaridenv.2014.10.007>.

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**Appendix E. First records and community pattern of Arcellinida inhabiting a
pristine and remote island from southeastern Pacific, Chile**

First Records and Community Pattern of Arcellinida Inhabiting a Pristine and Remote Island from Southeastern Pacific, Chile

Leonardo David FERNÁNDEZ^{1,2,3}, Jaime ZAPATA², Ralf MEISTERFELD⁴ and Luisa BAESSOLO⁵

¹Department of Zoology, Faculty of Natural and Oceanographic Sciences, University of Concepcion, Concepción, Chile; ²Center for Studies in Chilean Biodiversity (CEBCH), Osorno, Chile; ³Sociedad Paleontológica de Chile (SPACH), Santiago, Chile; ⁴Institute for Biology II, Unit Cellular Neurobiology, RWTH Aachen University, Aachen, Germany; ⁵Programa de Educación e Investigación Biológica & Ambiental (Programa-IBAM), University of Los Lagos, Osorno-CHILE

Summary. We investigate for the first time the species composition and community structure of lobose thecamoebians (Arcellinida) inhabiting an unpopulated and pristine island from the southeastern Pacific. Results revealed low alpha diversity and a high proportion of cosmopolitan species. One genus, four species and two subspecies were identified for the first time for southwestern South America. Further, four morphotypes were not identified to species level, and one could not be identified to species or genera level. They are probably endemics of this poorly studied and remote zone. These results were consistent with the moderate endemism hypothesis of microbial biogeography. We hypothesized that the low diversity of species recorded on the island is due to selective colonization-extinction dynamics, processes that determines the low species richness of insular macro-organisms. However, this hypothesis needs to be evaluated in the future. Statistical analysis showed that testate amoebae were distributed in two discrete communities in the island. The first consisted of organisms inhabiting habitats located within a forest and the second by organisms inhabiting habitats located outside the forest. The suggested primary factor differentiating these both communities was the availability of appropriate habitat for the different species of testate amoebae.

Key words: Chile, Guamblin Island National Park, lobose testate amoebae, North Patagonian rainforest, southwestern South America.

INTRODUCTION

In opposition to the idea that all the microorganisms have a cosmopolitan or ubiquitous distribution, many

studies support the hypothesis that some free-living protists have biogeographic patterns (e.g. see Foissner and Hawksworth 2009 or Fontaneto 2011 for many examples). However, if we compare the number of investigations on protists made in the southern hemisphere with those made in the northern hemisphere, it is easy to conclude that the former has been historically less studied (Fenchel and Finlay 2003, Mitchell and Meisterfeld 2005). Consequently, the general approval for this hypothesis has been undermined mainly by the lack of data from unexplored regions of the planet. This lack

Address for correspondence: Leonardo D. Fernández-Parra, Programa de Doctorado en Sistemática y Biodiversidad, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción-CHILE. Tel. (+56) 41 220 7325; Fax (+56) 41 23 8982; E-mail: limnoleo@gmail.com

of information on protists is especially evident in southwestern South America, Chile, a region where research on biota is strongly biased towards vertebrates (Simionetti *et al.* 1995). Particularly, remotes areas of the southern Chilean coastline, archipelago and fjordland (41–56°S) are amongst the most poorly studied areas of the planet (Schrödl and Grau 2003, Arriagada 2010); and except for a number of recent studies on foraminifera (e.g. Zapata 1999; Hromic 2002, 2009; Fernández 2010; Fernández and Zapata 2010a, b), free-living protists belonging to these remote areas remain still unknown. Moreover, most of the studies carried out on testate amoebae in Chile correspond to taxonomic reports (Fernández and Zapata 2011); as a result, little is known on the community pattern of these microorganisms in southwestern South America.

In order to reduce the gap in the global knowledge of these microorganisms, this study presents the first records and community patterns of testate amoebae (Arcellinida) inhabiting the southern Chilean Guamblin Island, one of the most remote and oceanic islands of Los Chonos Archipelago. The outcomes supplied by this study could provide valuable antecedents to the current debate on the biogeography of protists.

MATERIALS AND METHODS

Study site

The Chonos Archipelago is made up of over 150 islands separated by saltwater channels and fjords scattered along the western margin of Aysén Region, southern Chile, between latitudes 44°S and 46°S (Haberle and Bennett 2004). The region lies within a zone of high precipitation, produced by the coupled ocean–atmospheric influence of the Southern Polar Front that migrates seasonally between 50°S (austral summer) and 45°S (austral winter) (Haberle and Bennett 2004). The climate is strongly oceanic, with annual precipitation in the region in the order of 3,000 mm. Annual average temperatures at sea level range between 8 and 10°C, and decrease further to the east with increasing continentality and with increasing altitude (Szeicz *et al.* 2003). Guamblin Island is the most oceanic of the islands composing the Chonos Archipelago and lies at about 83 nautical miles from the mainland. This island has an area of 15,912 ha and a maximum attitude of 218 m. In 1967 a National Park was designated in order to protect both the biodiversity and the purity of its landscapes (CONAMA 1987). Guamblin Island is an excellent natural laboratory to test the dispersal capability of protist since it is an extremely inaccessible and pristine mass of land, without any anthropogenic intervention; nobody lives or have lived on the island in the past (it is completely uninhabited); has many habitats similar to those found in the nearby mainland (e.g. North Patagonian rain-forest, creeks, swamps), which in turn, are potentially suitable for

the establishment of the arcellinids; and naturally, is isolated. These precedents lead us to assume that the occurrence of all of its biotic components can be attributed to natural processes and to consider a minimal (if any) anthropogenic influence on the dispersal mechanism and subsequent establishment of protists on island.

Sampling of testate amoebae and laboratory treatment

Fifteen surface samples (1,000 cm³) were collected in January 2008 (austral summer) along a ca. 5,000 m longitudinal transect, covering the northeastern side (closest area to the mainland) of the Guamblin Island (Fig. 1). We sampled only this area because the remainder of the island was, at that time, totally inaccessible by foot or by sailing (trekking through the forest was almost impossible, and there are many cliffs and rocky outcrops along the coastline that do not allow a safely disembarkation). All samples were collected in potential habitats for these microorganisms, but bryophytes (a classical habitat for testate amoebae) were absent along this transect. Details of the sampling sites are given in Table 1 and two representative examples are shown in Fig. 2. All samples were stored wet to maintain the original moisture conditions during transport and were studied within three weeks after collection. The samples were maintained at air temperature (the austral summer is 12°C on average at this latitude) and were protected from direct sunlight during the whole stay on the island and the return trip. On return to the laboratory, samples were washed through 500, 250 µm mesh sieves to retain the coarse organic material, and then back-sieving over a 30 µm mesh sieve to retain microorganisms. The resulting filtrate of each sample was deposited on a Petri plate and air-dried at room temperature. All samples were examined under a light microscope at high magnification and all individuals were isolated and counted. The determination of species was based on morphological characters of the shell (morphospecies), according to a wide range of literature. Important sources used in the determinations included those of Certes (1889), Wailes (1913), Jung (1942), Bonnet (1966), Meisterfeld (2002) and Zapata *et al.* (2007). For scanning electron microscopy, the organisms (shells) were isolated, mounted on coverslips and finally air-dried. The shells on coverslips were coated with gold and examined with a JEOL JSM-6380 operating at 20 kV.

Evaluation of sampling artifacts

In order to rule out possible artifacts on sampling effort we performed a species-accumulation curve method. Species-accumulation curve take account of the identity of the species and plot the rate of accumulation of the new species sampled as samples of identical size are pooled over the total area sampled (Ugland *et al.* 2003). Because all communities contain a finite number of species, if the surveyors continued to sample, the curves would eventually reach an asymptote at the actual community richness. Thus, the curves contain information about how well the communities have been sampled (i.e. what fraction of the species in the community have been detected) (Soberón and Llorente 1993). Here, we use the non-parametric abundance-based estimator Chao1 to estimate richness (Chao 1984, Colwell and Coddington 1994). Species accumulation curve was calculated with EstimateS v8 software (Colwell 2012) using 1,000 randomizations and sampling without replacement. To see if result was sensitive to choice of richness estimator, we also ran selected analyses using a number of other common non-para-

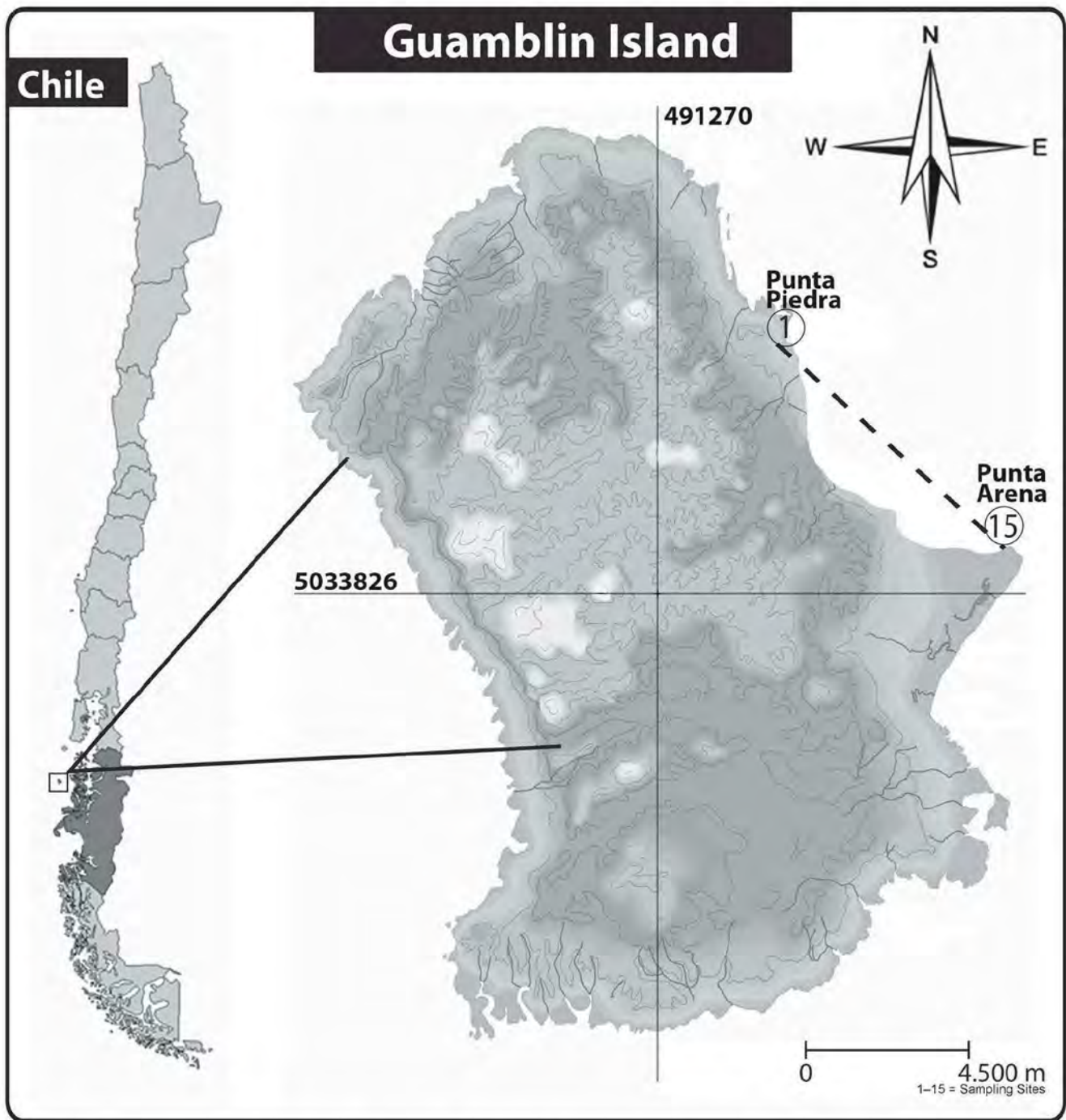


Fig. 1. Map of the Guamblin Island National Park. Samples were taken at equidistant distances, ranging from sector Punta Piedra (sampling site number 1) to sector Punta Arena (sampling site number 15).

metric estimators based in both incidence and abundance of species (i.e. Jack1, Jack2, Chao2, ICE and ACE).

Multivariate analyses

Individuals not identified to genera or species level were excluded from multivariate analyses. Although they may belong to new taxa, we must still confirm that they are not just aberrant

individuals. In addition, we lumped together all subspecies (formerly considered as varieties) in their respective morphospecies, since many of them have not been confirmed for anyone else than the person who first described (Mitchell and Meisterfeld 2005), situation very common for this region of the globe. These procedures were performed to reduce skewed or distorted results. Then, we fourth root transformed data to down-weight the influence of

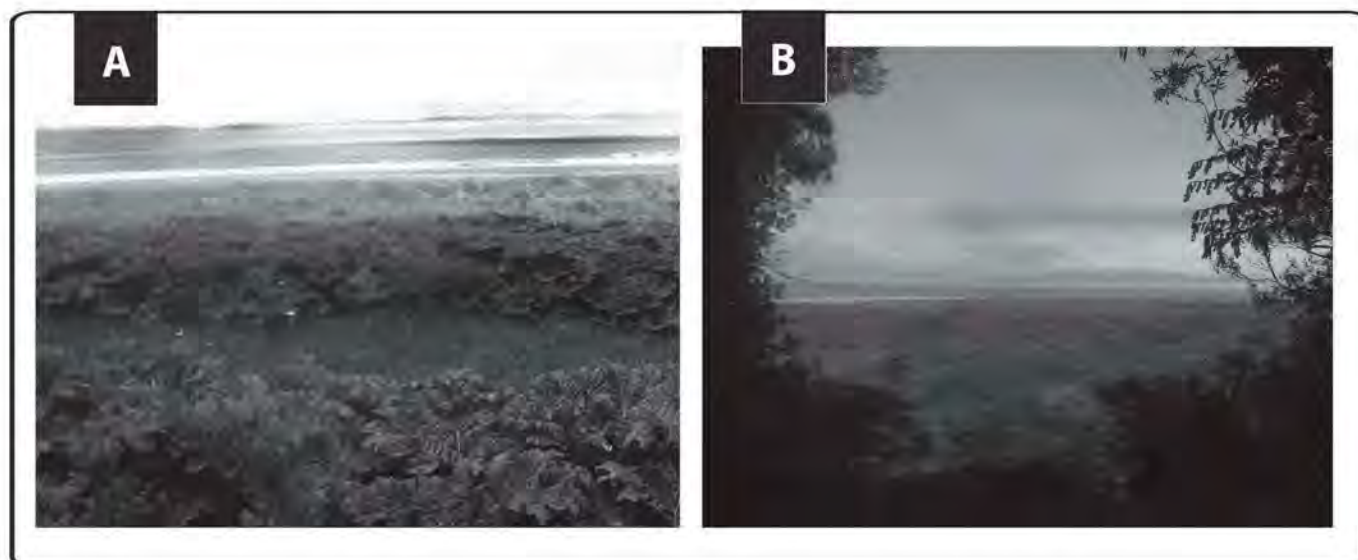


Fig. 2. Both images correspond to the northeastern side of the Guamblin Island ('closest' area to mainland). These images are useful to show the contrasting nature of the two types of sampled environments (treeless and forested environments). **A** – this photo corresponds to the sampled coastal swamp, which is located in sector Punta Piedra (see Fig. 1). This environment is dominated by *Gunnera* spp. and *Juncus* sp.; **B** – this photo corresponds to sector Punta Arena (see Fig. 1), and was taken from an elevation of 150 m a.s.l. In the foreground are some ferns (*Lomatia ferruginea*) and trees (*Nothofagus* spp., *Amomyrtus luma* and *Maytenus boaria*). In the background is the North Patagonian rain forest (which is mainly dominated by *Nothofagus* spp.) and towards the end the Southeastern Pacific. Photo credits by J. Pérez.

Table 1. Description of sampling sites surveyed on Guamblin Island. See some global examples in Fig. 2.

Sampling sites	Habitat	General remarks
S1–S2	Creeks	Aquatic soil from two small creeks located outside the forest, close to a swamp and surrounded by marsh vegetation
S3	Swamp	Coastal swamp, located very close to sea, dominated by <i>Gunnera</i> spp. and <i>Juncus</i> sp. (probably <i>J. procerus</i>)
S4	Swamp	Swamp located at forest margins, dominated by <i>Gunnera</i> spp., <i>Baccharis linearis</i> , <i>Blechnum chilense</i> and <i>Juncus</i> sp. (probably <i>J. procerus</i>)
S5	Cave	Mud from a little cave located at forest margins, without vegetation.
S6, S7, S9, S12–S15	Forest	Litter and soil from sites located within North Patagonian rain forest, dominated mainly by <i>Nothofagus dombeyi</i> and <i>N. nitida</i>
S8, S10, S11	Creek	Three small creeks located within North Patagonian rain forest and surrounded mainly by <i>Gunnera</i> spp., <i>B. chilense</i> and <i>Drimys winteri</i>

abundant taxa and account for rare taxa as well (Kreutzweiser *et al.* 2005). Cluster analysis (group average method on Bray–Curtis similarity index) was used to quantify community differences and similarities between environments sampled. To confirm that the groups (i.e. communities) created by the cluster analysis were natural, we subjected the similarity matrix to a 2-d non-metric multidimensional scaling (nMDS), which is a robust ordination method for ecological community data (Clarke and Gorley 2005). In order to identify significant groupings of testate amoebae according to species composition (i.e. deviated from null structure at $\alpha = 0.05$) we performed a similarity profile (SIMPROF) analysis

(Clarke 1993). Here, each of the variables (species) was randomized across samples (sampling sites) and the rank order of similarities recalculated over 1,000 permutations. Similarity of percentage (SIMPER) analysis, with a cut off for low contributions of 50%, was used to determine the percentage contribution of individual species towards describing the communities of testate amoebae by species composition (Clarke 1993). Bubble-plots of the most conspicuous species were selected because they were identified as primary factor differentiating the major communities and were superimposed onto nMDS plots. All the analyses were performed in PRIMER v6 software (Clarke and Gorley 2005).

RESULTS

A total of 18,620 thecamoebians, distributed in 12 genera, 33 species and five subspecies were identified and counted from Guamblin Island. One genus, four species and two subspecies are mentioned for first time for southwestern South America (Table 2). On the other hand, four morphotypes were not identified to species level, and one could not be identified to species or genera level (Table 2). We do not make a formal description of these taxa, because the number of individuals per sample was too low to confirm that they are not just aberrant individuals. Except for these individuals, all of testate amoebae identified in the present study have been recorded previously in other regions of the world, including continental Chile (i.e. cosmopolitan or ubiquitous microorganisms) (Fig. 3).

The most diverse genera were *Centropyxis* and *Diffugia*, with five and seven species correspondingly (Table 2). In addition, the five most abundant species (taxa with > 5% of total individuals) made up 56.1% (10,460 individuals) of the total testate amoebae recorded in the Guamblin Island (Table 2). These taxa encompassed *Centropyxis aculeata* 32.5% (6,050 individuals, lumping all the subspecies as one taxon), *Apodera vas* 6.8% (1,260 individuals), *Centropyxis elongata* 5.7% (1,070 individuals), *Cyclopyxis arcelloides* 5.7% (1,060 individuals), and *Centropyxis aerophila* 5.5% (1,020 individuals) (Table 2). Moreover, our species-accumulation curve was close to saturation, showing that our sampling effort provides a reliable species richness for the sampling sites, since all the data points estimated with Chao1 fall within the 95% probability intervals (Fig. 4). Estimation of species richness was not changed when we explored the use of species richness estimators other than Chao1. Indeed, species-accumulation curve using Jack1, Jack2, Chao2, ICE and ACE produced estimations that also are within the 95% probability intervals given by Chao1 analysis (results are not shown).

The Cluster and SIMPROF analyses identified two broad and statistically significant groups (i.e. two communities, $p = 0.001$, Fig. 5). The first of these groups (community A) consisted of samples taken exclusively outside the forest (creeks and swamps, S1–S4), which in turn, was subdivided into two statistically significant smaller clusters ($p < 0.01$, Fig. 5). In contrast, the second group (community B) was composed of samples taken both within and at forest margins (forest soil, creeks and cave, S5–S11), which was subdivided into two

significant clusters: mud from a little cave and the rest ($p < 0.01$, Fig. 5). The nMDS ordination analysis also supported graphically the existence of these two broad and discrete communities (Kruskal stress = 0.13, Fig. 6).

The SIMPER analysis revealed that the average Bray–Curtis similarity between all pairs of sampling sites in the community A was 60.44%, made up mainly of contributions from four species (Table 3, columns 2 and 4). Among these were the species *C. aculeata*, *Cy. arcelloides*, *Diffugia oblonga curvicollis* and *D. globularis* with a cumulative contribution of about 50% of the total similarity of 60.44% (Table 3, column 5). Furthermore, the community B obtained a within-group average Bray–Curtis similarity of 63.91%, made up mainly of contributions from five species (Table 3, columns 2 and 4). *C. aculeata* was again prominent among these species, but the remaining four species (*Argygnia dentistoma*, *A. vas*, *Certesella martiali*, *C. aerophila*) were completely different from those listed as major contributors in the community A. These five species contributed with about 50% of the total similarity of 63.91% (Table 3, column 5). We bubble-plotted onto nMDS plots the two most conspicuous species making up the observed similarity within each community in Fig. 7 (excluding a priori *C. aculeata*, since was present in both communities).

Additionally, the SIMPER analysis also found that the average of the Bray–Curtis dissimilarities between both communities was 56.31%. Ten species accounted for more than 50% of average dissimilarity between both communities (Table 4). For instance, this dissimilarity was made up of 4.45% from *Cy. arcelloides*, 3.94% from *D. globularis* and so on (Table 4, column 3). The *Cy. arcelloides* contribution was 7.55% of the total of 56.31%, *D. globularis* gave 6.99% of this total (Table 4, column 5). These values percentages were accumulated in the column 6 (Table 4), until the cut-off of > 50% was reached. The ratio of the average contribution/standard deviation (column 4) was always high for each of these ten species. We bubble-plotted onto nMDS plots the two most conspicuous species making up the observed distinction between both communities (Fig. 8).

DISCUSSION

The so-called ‘ubiquitous dispersal hypothesis’ states that all microorganisms can be found anywhere on the planet as long as suitable conditions are met

Table 2. Occurrences of testate amoebae in each sampling site on Guambin Island, Southern Chile (individual abundances were divided by the total for that sample).

	Samples														
	Community A							Community B							
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
<i>Apodera vas</i> Certes	0.0	0.0	1.9	4.3	6.8	7.8	11.7	4.8	19.6	4.0	0.0	12.4	7.9	13.7	31.3
<i>Argygnia dentistoma</i> Penard	0.0	0.0	1.0	3.5	4.5	4.9	7.8	4.8	4.7	6.0	9.1	11.3	6.3	3.8	10.0
<i>Argygnia vitrea</i> Penard	0.0	0.0	0.5	4.3	6.8	3.9	0.0	0.0	0.0	5.0	4.5	0.0	7.1	7.6	6.3
<i>Centropyxis aculeata aculeata</i> (Ehrenberg)	15.6	15.3	36.3	47.8	36.4	42.2	16.9	54.2	13.1	45.0	47.8	18.5	35.4	30.5	15.0
<i>Centropyxis aculeata oblonga</i> (Ehrenberg)	5.5	8.3	0.0	0.0	1.1	2.9	3.9	2.4	2.8	2.0	0.0	0.0	2.4	3.8	0.0
<i>Centropyxis aerophila</i> Deflandre	10.1	8.7	0.0	4.3	0.0	11.8	7.8	2.4	4.7	8.0	3.4	3.1	4.7	6.9	0.0
<i>Centropyxis constricta</i> (Ehrenberg)	6.9	8.3	0.0	0.0	5.7	7.8	3.9	0.0	0.0	0.0	5.7	3.1	6.3	2.3	2.5
<i>Centropyxis discoides</i> Penard	9.6	7.9	1.0	0.0	0.0	0.0	0.0	0.0	4.7	8.0	6.8	3.1	0.0	0.0	10.0
<i>Centropyxis elongata</i> (Penard)	6.9	3.3	1.4	0.0	26.1	7.8	2.6	0.0	0.0	0.0	5.7	20.6	9.4	8.4	0.0
<i>Certesella martiali</i> (Certes)	0.0	0.0	1.0	0.0	1.1	2.0	10.4	3.6	24.3	2.0	0.0	12.4	4.7	3.8	10.0
<i>Cyclopyxis arcelloides</i> (Penard)	9.6	8.3	24.9	5.2	0.0	0.0	0.0	0.0	0.0	0.0	5.7	0.0	1.6	0.0	0.0
<i>Cyclopyxis intermedia</i> * Kufferath	2.3	0.8	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cyclopyxis kahli</i> Deflandre	3.7	0.0	0.0	0.0	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cyclopyxis eurystoma</i> Deflandre	2.8	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cyclopyxis</i> sp.	0.9	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Diffugia bryophila</i> Penard	0.0	1.2	0.0	0.0	0.0	2.0	15.6	2.4	14.0	3.0	0.0	2.1	6.3	5.3	6.3
<i>Diffugia cylindrus</i> * (Thomas)	7.3	12.8	11.0	0.0	0.0	2.0	5.2	2.4	4.7	3.0	0.0	0.0	0.0	0.0	0.0
<i>Diffugia globularis</i> Wallich	0.9	11.6	7.2	4.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Diffugia lanceolata</i> * Penard	2.3	1.2	4.8	1.7	1.1	0.0	1.3	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
<i>Diffugia mitriformis</i> Wallich	2.3	0.8	3.8	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Diffugia oblonga curvicolis</i> ** Ehrenberg c.f.	8.3	9.1	2.4	7.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0	0.0
<i>Diffugia lata</i> * (Ehrenberg)	1.8	1.2	1.0	0.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Heleopera petricola</i> Leidy	0.9	0.8	0.0	0.0	0.0	0.0	2.6	3.6	0.0	0.0	2.3	0.0	0.0	0.8	0.0
<i>Heleopera sphagni</i> (Leidy)	0.0	0.0	0.0	0.0	0.0	2.0	2.6	1.2	0.0	0.0	0.0	3.1	1.6	1.5	0.0
<i>Lagenodiffugia</i> sp.	0.0	0.0	1.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nebela barbata psilonata</i> Leidy	0.9	0.4	0.0	0.9	2.3	2.0	2.6	6.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0
<i>Nebela collaris</i> (Ehrenberg)	0.0	0.0	0.0	1.7	0.0	0.0	2.6	6.0	2.8	2.0	5.7	3.1	0.8	1.5	6.3
<i>Nebela penardiana</i> Deflandre	0.0	0.0	0.0	1.7	0.0	1.0	2.6	0.0	4.7	3.0	0.0	2.1	3.1	2.3	2.5
<i>Nebela</i> sp.1	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0
<i>Nebela</i> sp.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0
<i>Padaungiella</i> (<i>Nebela</i>) <i>lageniformis</i> (Penard) c.f.†	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	2.0	0.0	5.2	0.0	4.6	0.0
<i>Plagiopyxis glyphostoma major</i> ** Bonnet	1.4	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pontigulasia compressa</i> Rhumbler	0.0	0.0	0.0	2.6	0.0	0.0	0.0	6.0	0.0	6.0	3.4	0.0	0.0	0.0	0.0
Undetermined individual	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* Species mentioned for first time for southwestern South America; ** subspecies mentioned for first time for southwestern South America; † formerly *Nebela lageniformis*, now transferred to new genus *Padaungiella* (Kosakyan *et al.* 2012).

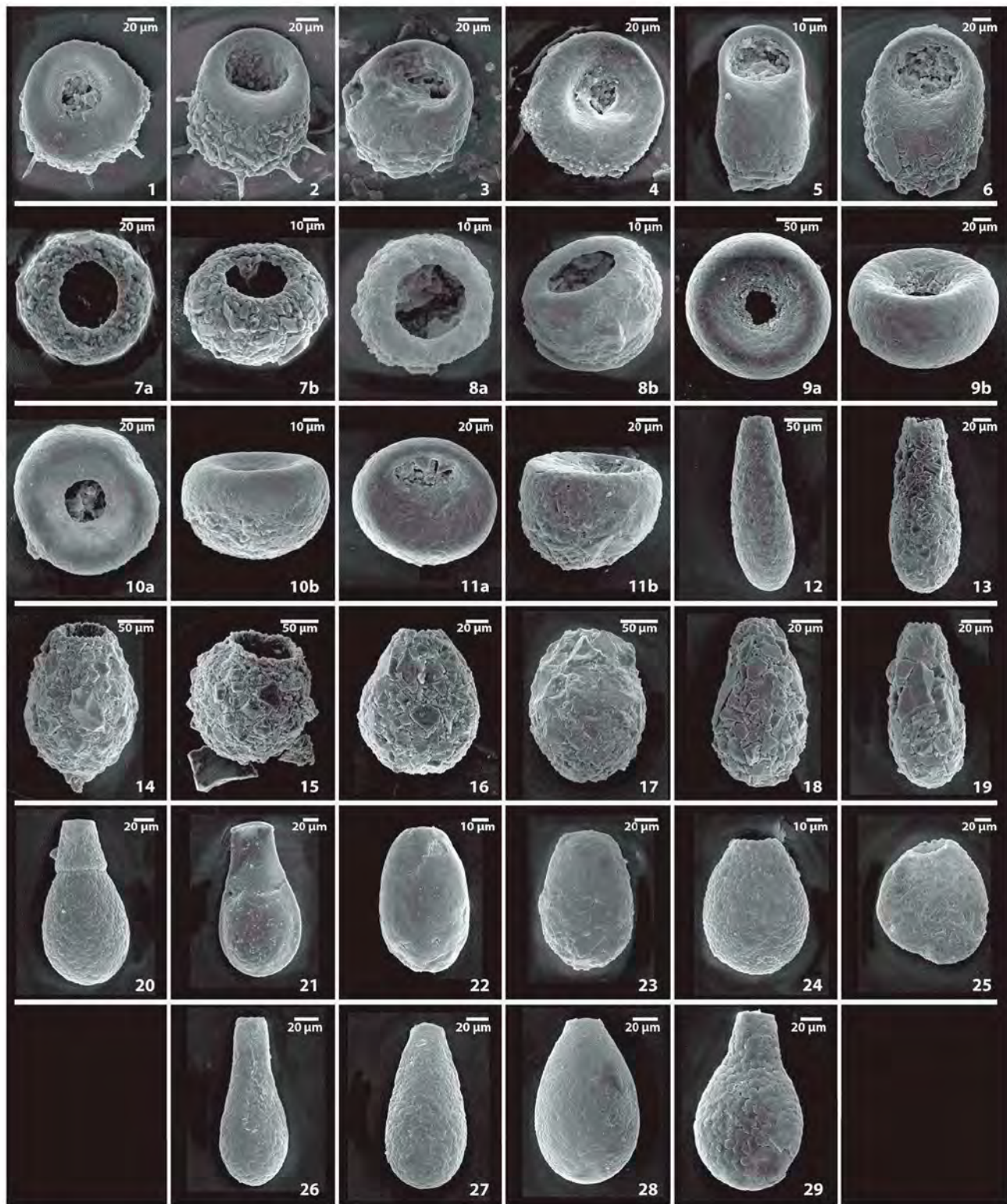


Fig. 3. Species observed on the Guamblin Island. 1 – *Centropyxis aculeata aculeata*; 2 – *C. aculeata oblonga*; 3 – *C. aerophila*; 4 – *C. discoides*; 5 – *C. elongata*; 6 – *C. constricta*; 7a, b – *Cyclopyxis arcelloides*; 8a, b – *C. eurystoma*; 9a, b – *C. intermedia*; 10a, b – *C. kahli*; 11a, b – *Plagiopyxis glyphostoma major*; 12 – *Diffugia lanceolata*; 13 – *D. cylindrus*; 14 – *D. mitriformis*; 15 – *D. globularis*; 16 – *Pontigulasia compressa* c.f.; 17 – *D. lata*; 18 – *D. oblonga curvicollis* c.f.; 19 – *D. bryophila*; 20 – *Apodera vas*; 21 – *Certesella certesi*; 22 – *Heleopera sphagni*; 23 – *H. petricola*; 24 – *Argynnia dentistoma*; 25 – *A. vitrea*; 26 – *Nebela barbata psilonata*; 27 – *N. penardiana*; 28 – *N. collaris*; 29 – *Padaungiella (Nebela) lageniformis*. The background of SEM images were retouched in some cases to highlight the organisms, however, the microorganisms *per se* were not manipulated in any way.

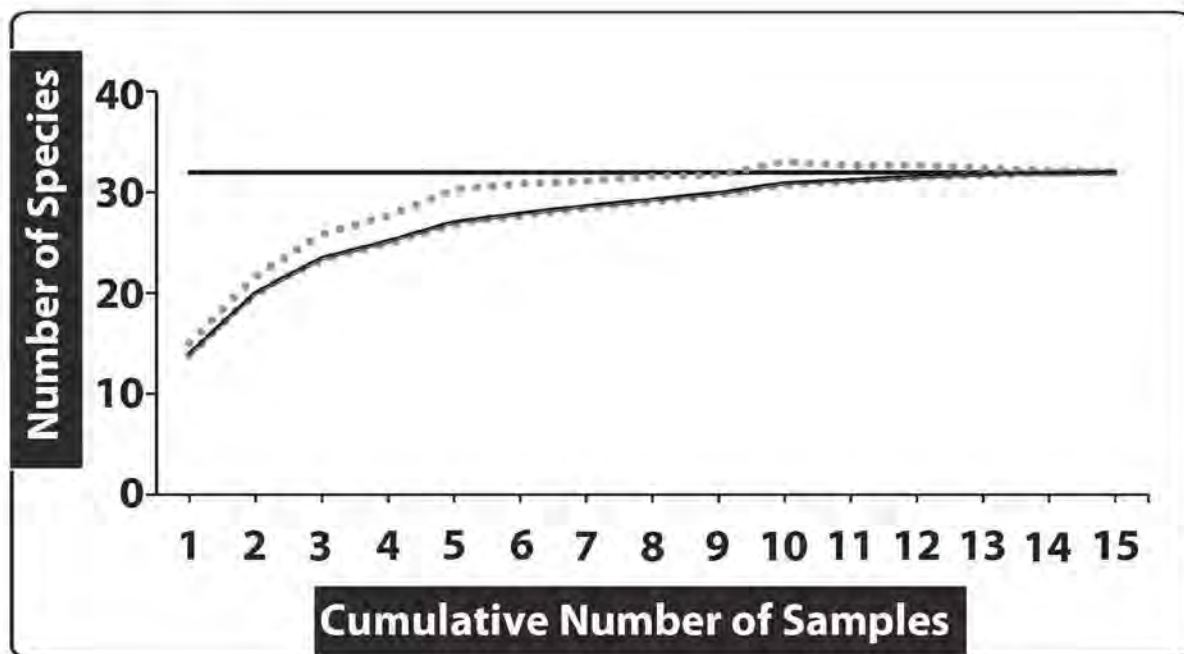


Fig. 4. Species-accumulation curve for testate amoebae collected in the Guamblin Island. Our observed number of species was 33 (black horizontal line). This value was consistent with the estimate species richness curve based on Chao1 (black irregular line). Upper and lower confidence intervals are shown as gray dotted lines (calculated using 1,000 permutations without replacement).

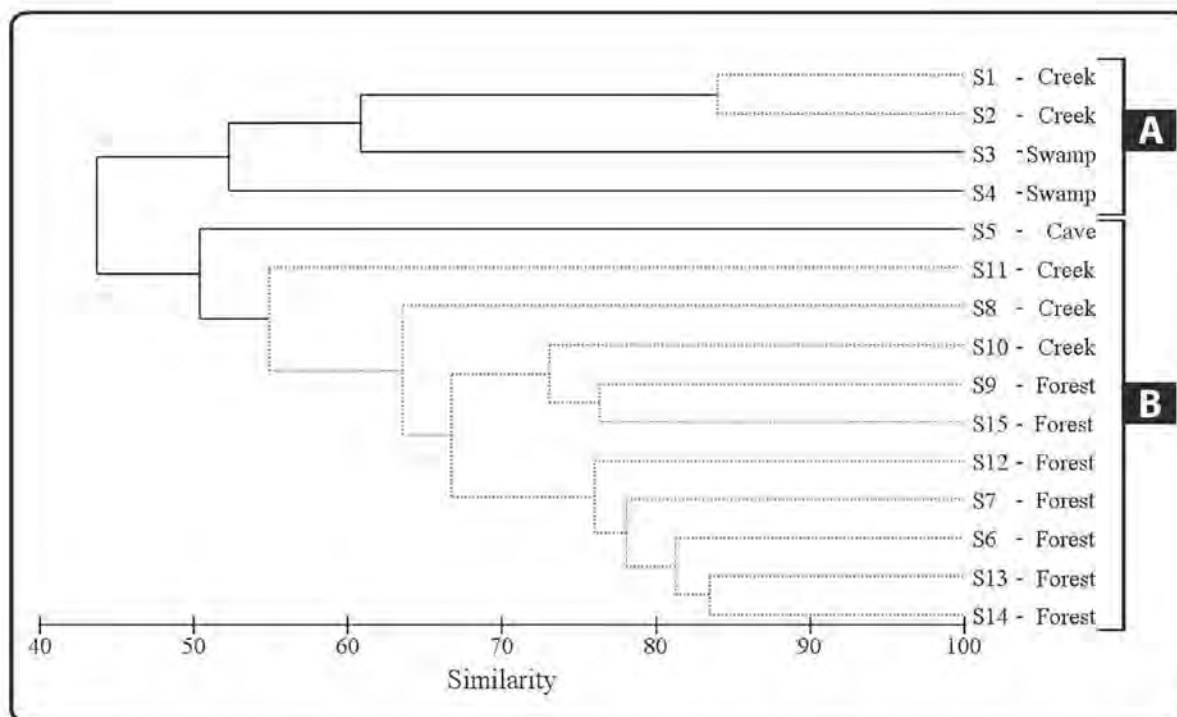


Fig. 5. Bracketed samples encompass groupings (i.e. communities) that are dissimilar from other sampling sites at 43.69% ($p = 0.001$). **A** – Community A, includes those sampling sites located outside the forest (treeless-group); and **B** – Community B, includes those sampling sites located within the forest and at the forest margins (forested-group). Groupings are according to the group average method on Bray–Curtis similarity index and fourth root transformed abundance-data. Black solid lines correspond to significant clusters and dotted lines correspond to clusters without significant internal structure (based on SIMPER analysis using 1,000 permutations).

(Fenchel and Finlay 2003, Fenchel 2005). Guamblin Island has many habitats similar to those found in the nearby mainland, which in turn, are potentially suitable for the establishment of the arcellinids. Nonetheless, our outcomes did not support the ubiquity hypothesis; because we recorded only a small fraction of the total species richness observed in the nearby mainland (33 species on the island versus more than 100 species of Arcellinida recorded on the near Chilean continent, Fernández unpublished data). Such situations are usually attributed to a low sampling effort (Fenchel and Finlay 2003), but our species-accumulation curve, based on Chao1 species richness estimator, showed that estimated curve reach an asymptote at the actual community species richness (Fig. 3), suggesting that only few more species of arcellinids are to be expected in future surveys. These results were concordant with the so-called ‘moderate endemism hypothesis,’ which proposes that at least some microorganisms have restricted geographical distribution (Foissner and Hawksworth 2009, Fontaneto 2011).

One of the leading causes discussed to explain why some protists have a limited distribution is the ‘shell size’ of these organisms (Wilkinson 2001, Wilkinson and Smith 2006, Yang *et al.* 2010, Lara *et al.* 2011, Wilkinson *et al.* 2012). This predicts that, for thecamoebians, limited geographical range becomes commoner above shell sizes of 100–150 μm , because it would be more difficult for larger microorganisms to become airborne and travel long distances than for the smaller ones (Wilkinson 2001). However, our results did not support this hypothesis, since we recorded many large species on the island (Table 2), such as *A. vas* and *C. discoides*, arcellinids with a mean size of 174 μm (± 25.1) and 160 (± 46.17) μm , respectively (Zapata and Fernández 2008, Lahr *et al.* 2008). In a series of recent papers Foissner (2006, 2007, 2008) argues that a minute size is not a good reason to be a widely distributed organism. He cites several cases of macroorganisms with limited geographical distribution (e.g. macrofungi, mosses, ferns, and flowering plants), that possess microscopic or very small dispersal stages. Based on these examples he sug-

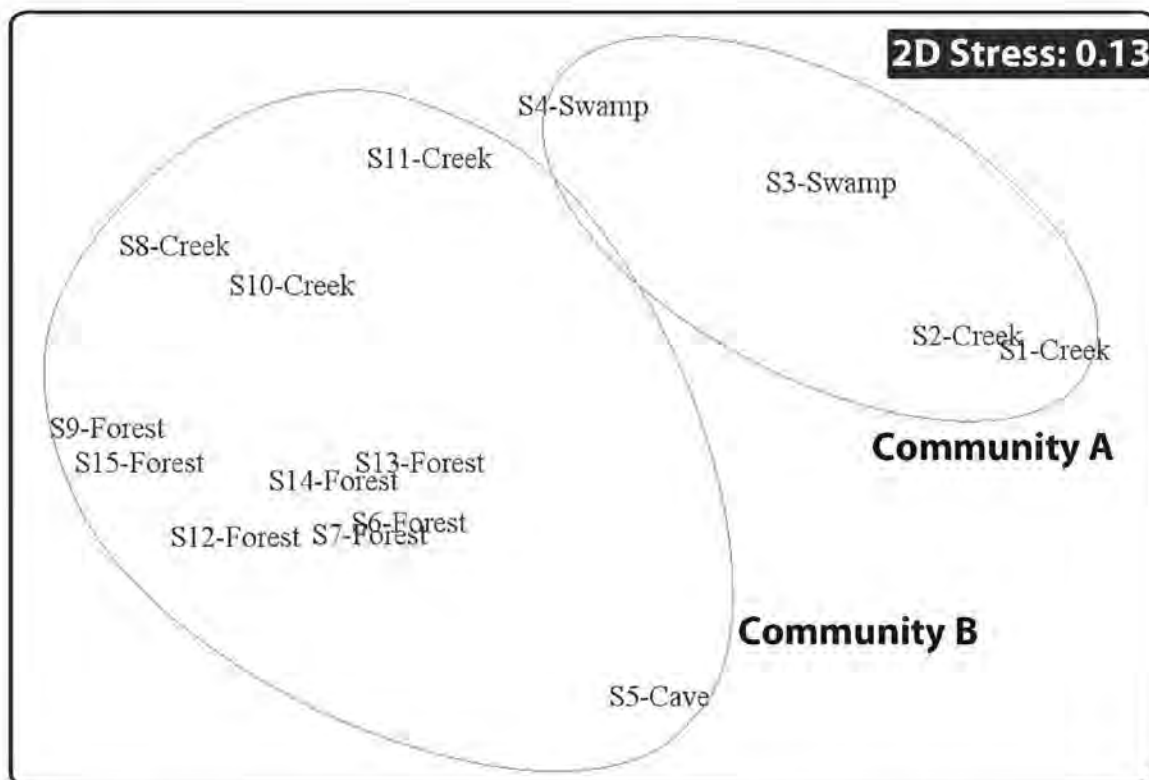


Fig. 6. Non-metric multidimensional scaling plot (nMDS) and Kruskal stress value for the nMDS configuration based on the abundance data of testate amoebae species found along the surveyed sampling sites on the Guamblin Island. Outlined circles represent groupings (i.e. communities) that are dissimilar from other sampling sites at 43.69% (based on SIMPER analysis using 1,000 permutations, $p = 0.001$). Groupings are according to the group average method on Bray–Curtis similarity index and fourth root transformed abundance-data.

Table 3. Species responsible for similarity within each community. The overall averages of the Bray–Curtis similarity (BC-s) between all sampling sites were 60.44% in the treeless-group (community A) and 63.91% in the forested-group (community B), respectively. Column 1 shows the average abundance for each species in each of the two communities. Columns 2 and 4 show the average and the percentage contribution respectively of each species to the BC-s. The column 3 corresponds to the ratio of the average contribution divided by the standard deviation. Column 5 comprises the cumulative percentage contribution to the BC-s, until the cut-off > 50% was reached.

Species	Average abundance*	Average similitude	Sim/SD	Percentage contribution	Cumulative percentage contribution
Community A					
<i>Centropyxis aculeata</i>	2.76	10.58	9.92	17.50	17.50
<i>Cyclopyxis arcelloides</i>	2.13	7.26	6.97	12.02	29.52
<i>Diffugia oblonga curvicollis</i>	1.94	6.90	13.82	11.41	40.93
<i>Diffugia globularis</i>	1.74	5.67	3.78	9.38	50.31
Other taxa	14.12	30.03	–	49.68	100.00
Total	22.69	60.44	–	100.00	–
Community B					
<i>Centropyxis aculeata</i>	2.36	11.38	7.78	17.80	17.8
<i>Argynnia dentistoma</i>	1.57	7.81	11.19	12.21	30.01
<i>Apodera vas</i>	1.63	6.80	1.99	10.64	40.65
<i>Certesella martiali</i>	1.39	5.49	1.91	8.59	49.25
<i>Centropyxis aerophila</i>	1.25	4.61	1.35	7.21	56.46
Other taxa	10.80	27.82	–	43.54	100.00
Total	19.00	63.91	–	100.00	–

* Values were fourth root transformed (see Materials and methods).

Table 4. Dissimilarity comparison between treeless- and forested-groups (communities A and B respectively). The overall average of the Bray–Curtis dissimilarities between all pairs of sampling sites (BC-d) was 56.31%. Columns 1 and 2 show the average abundance for each species in each of the two communities. Columns 3 and 5 show the average and the percentage contribution respectively, of each species to the BC-d. The column 4 corresponds to the ratio of the average contribution (column 3) divided by the standard deviation. The column 6 comprises the cumulative percentage contribution to the BC-d, until the cut-off > 50% was reached.

Species	Average abundance* Community A	Average abundance* Community B	Average dissimilitude	Diss/SD	Percentage contribution	Cumulative percentage contribution
<i>Cyclopyxis arcelloides</i>	2.13	0.24	4.25	2.65	7.55	7.55
<i>Diffugia globularis</i>	1.74	0.00	3.94	3.74	6.99	14.54
<i>Diffugia oblonga curvicollis</i>	1.94	0.22	3.86	3.34	6.86	21.40
<i>Diffugia mitriformis</i>	1.34	0.00	3.02	4.47	5.37	26.76
<i>Diffugia cylindrus</i>	1.64	0.60	2.98	1.65	5.30	32.06
<i>Diffugia lanceolata</i>	1.44	0.27	2.66	2.21	4.72	36.78
<i>Certesella martiali</i>	0.30	1.39	2.55	1.73	4.53	41.32
<i>Centropyxis discoides</i>	1.35	0.70	2.38	1.43	4.23	45.55
<i>Diffugia bryophila</i>	0.33	1.23	2.34	1.48	4.15	49.70
<i>Centropyxis constricta</i>	1.02	1.04	2.30	1.50	4.09	53.79
Other taxa	12.16	13.61	26.02	–	46.21	100.00
Total	25.39	19.30	56.30	–	100.00	–

* Values were fourth root transformed (see Materials and methods).

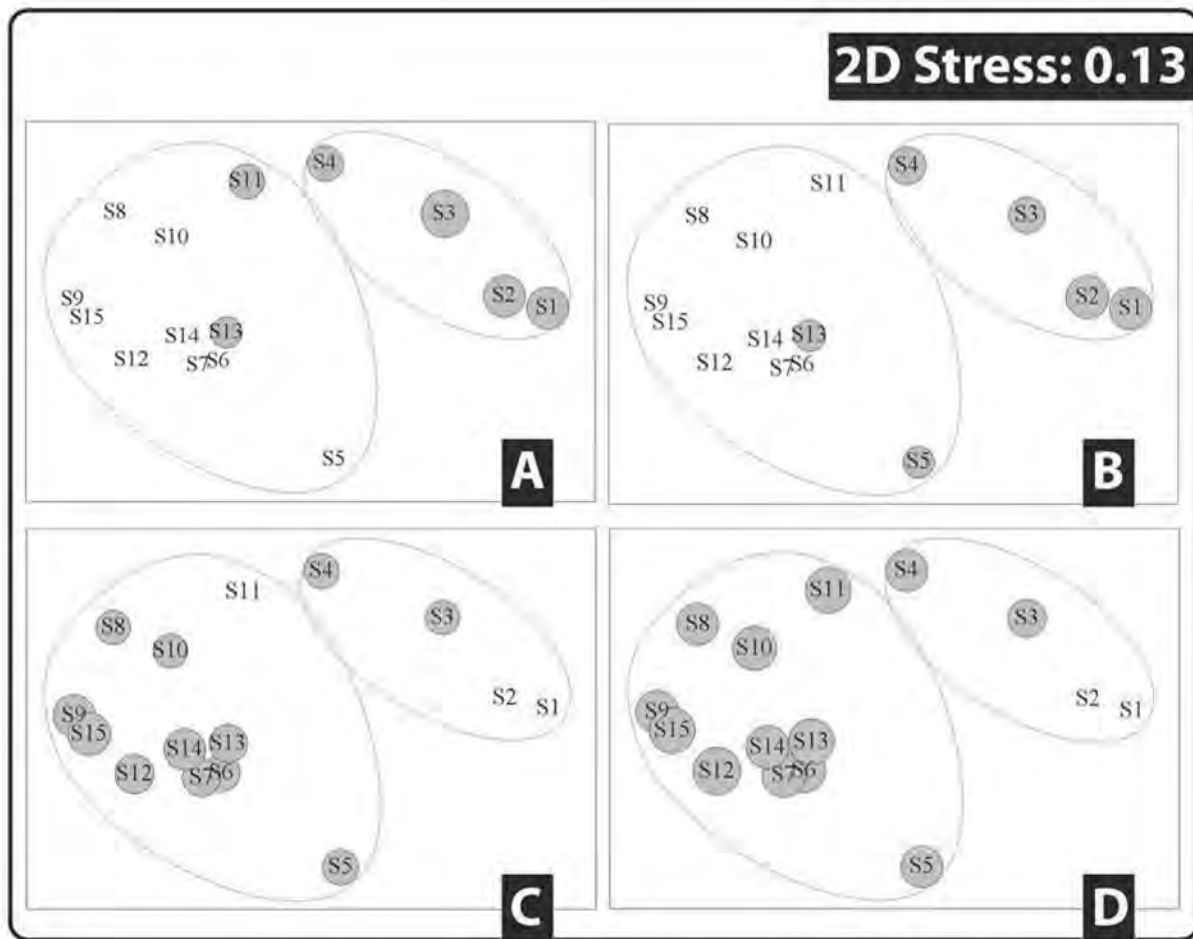


Fig. 7. Bubble plots of the two most conspicuous species making up the observed similarity within each community. For community A: **A** – *Cyclopyxis arcelloides*; **B** – *Diffugia oblonga curvicollis*; and for community B: **C** – *Argynnia dentistoma*; **D** – *Apodera vas*. Bubble plots are superimposed from the nMDS showed in Fig. 5. Bubble size approximates relative proportion of a given species in each sampling sites (gray circles with numbers) and each community type (outlined circles).

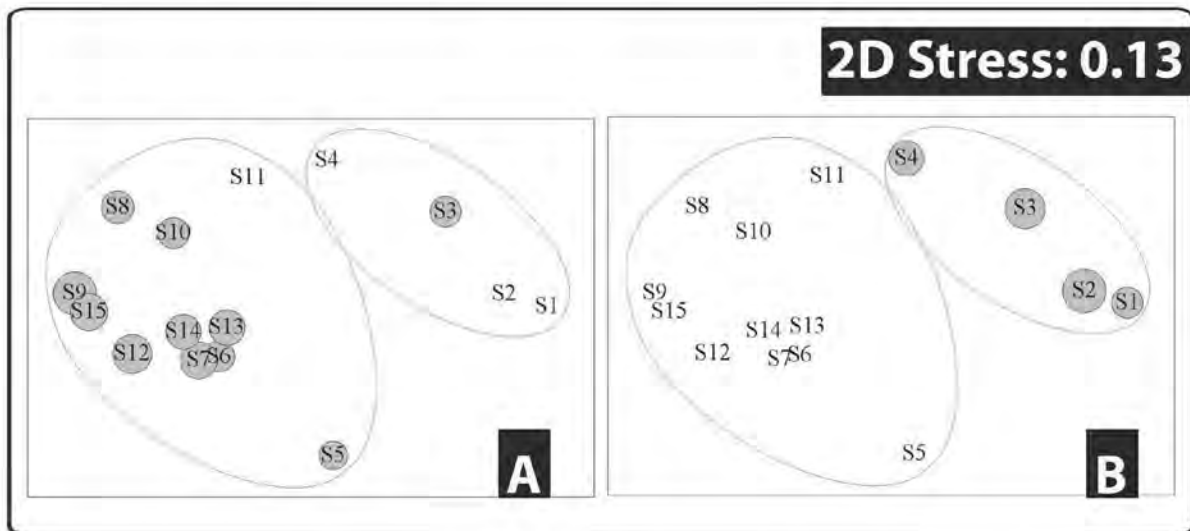


Fig. 8. Bubble plots of the two most conspicuous species making up the observed dissimilarity between both communities: **A** – *Certesella martiali* and **B** – *Diffugia globularis*. Bubble plots are superimposed from the nMDS showed in Fig. 5. Bubble size approximates relative proportion of a given morphospecies in each sampling sites (gray circles with numbers) and each community type (outlined circles).

collection and counting (including the abundances of *A. vas* and *C. martiali*). Nonetheless, when the aim is to measure abundances and species composition of the active species at the time of sampling (as in this paper), then, samples must be kept under conditions similar to the sample temperature and moisture (Adl and Gupta 2006; Bamforth 2007, 2010). Instead, the common method of air-drying samples for transport can lyse cells (Adl and Gupta 2006), changing irremediably the original species composition and abundances. Another recommendation is to examine the samples as soon as possible (Adl and Gupta 2006, Bamforth 2010), but given the remote location of the Guamblin Island, we were able to check samples only after three weeks. But still, we suggest that the time elapsed do not affected in a significant way the species composition and structure, because we recorded centropyxids, cyclopyxids and difflugids in high numbers within swamps and creeks, as is commonly reported in other studies conducted on these habitats (e.g. see Asada and Warner 2009 and references cited therein). Similarly, we recorded a high proportion of acrostomic species (e.g. nebelids *sensu* Meisterfeld 2002) within forest soils, as also is usually reported in similar researches (Bonnet 1966; Bamforth 2007, 2010; Krashevskaya *et al.* 2007; Fernández and Zapata 2011). In other words there was not abnormality in the community parameters except for a slightly higher abundance than usual for *A. vas* and *C. martiali*.

CONCLUDING REMARKS

Here, we investigate for the first time the species composition and community structure of testate amoeba inhabiting an unpopulated and pristine island from the southeastern Pacific. Our results revealed low alpha diversity and a high proportion of cosmopolitan species. Nonetheless, one genus, four species and two subspecies were recorded by first time, increasing to ~225 species the total number of testate amoebae recorded in southwestern South America (considering both filose and lobose thecamoebians). Moreover, four morphotypes were not identified to species level, and one could not be identified to species or genera level. This suggests that in past this island could be a glacial refugium, allowing the radiation of new testate amoebae with limited capacity of dispersion, since they had never been recorded before in the next mainland, although this zone of the Southamerican continent has been ex-

tensively studied during the last 30 years. But, at the same time, our results highlight the natural-high capacity of dispersal of cosmopolitan species, given that they are occurring on a uninhabited island, a place where (based on existing background) human influence is certainly very limited. However, the determination of these cosmopolitan species was not always easy using European descriptions. Maybe, in future, the use of the term 'cosmopolitan' would not apply to a wide fraction of the Chilean testate amoebae fauna. Upcoming molecular-based studies could help both to evaluate the genetic diversity within these microorganisms and elucidate this issue. We also hypothesized that the low diversity of species recorded on the island is due to dynamics of selective colonization-extinction, processes that determines the low species richness of insular macro-organisms. However, this hypothesis needs to be evaluated in the future. Further, we show that testate amoebae are distributed in two discrete communities along the studied transect. The first one (community A) was constrained to habitats located outside the forest and had soil and aquatic testate amoebae as typical species. The second one (community B) was restricted to habitats located within the forest and had wet forest soils testate amoebae as typical species. The suggested primary factor differentiating these both communities was the availability of appropriate habitat for the different species of testate amoeba. For instance, the lack of persistent aquatic environments within the forest probably limited the occurrences of difflugids in community B and the prevalent harsh conditions (e.g. occasional flooding from the sea) outside the forest restricted the occurrences of nebelids in community A. Finally, we stress the prevalent occurrence of *A. vas* and *C. martiali* within the Nord Patagonian rainforest. The observed occurrences of both nebelids in this island and another pristine environments suggest that the prevalence of these two species could be used as an indicator of pristine environmental conditions. Nonetheless, further studies are necessary to confirm this.

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Appendix F. Variación estacional en la comunidad de amebas testadas de una turbera temperada del sur de Chile

VARIACIÓN ESTACIONAL EN LA COMUNIDAD DE AMEBAS TESTADAS DE UNA TURBERA TEMPERADA DEL SUR DE CHILE

Seasonal variation in the testate amoebae community of a temperate
peatland from Southern Chile

LEONARDO FERNÁNDEZ P.^{1,2,3} & JAIME ZAPATA M.¹

¹ Centro de Estudios en Biodiversidad (CEBCh), Magallanes 1979, Osorno, Chile.

² Programa de Doctorado en Sistemática y Biodiversidad, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile.

³ Becario de la Comisión Nacional de Investigación Científica y Tecnológica (CONICYT), Chile. E-mail: limnoleo@gmail.com

RESUMEN

En la actualidad existe un gran número de trabajos que documentan la relación existente entre las amebas testadas y los parámetros ecológicos que las controlan en el tiempo. No obstante, esta información es muy escasa cuando nos referimos a la región temperada del sudeste americano. Esto es el principal impedimento para la implementación de estos organismos como bioindicadores de parámetros físico-químicos, ecológicos, sedimentológicos y paleoecológicos en estas latitudes. En consecuencia, este trabajo tiene por finalidad: 1) determinar la variación estacional en la composición de la comunidad de amebas testadas de una turbera temperada dominada por musgo *Sphagnum*; 2) analizar el significado ecológico de esta variación; 3) inferir la influencia del pH y la temperatura en la distribución de las tecombeas y 4) entregar antecedentes para posteriores estudios paleoecológicos en las turberas temperadas de Chile.

Palabras Claves: Amebas testadas. Turbera temperada. Variación estacional. Región de Los Lagos. Chile.

ABSTRACT

Currently there are a large number of papers documenting the relationship between testate amoebae and their ecological controls along the time. However, this information is very scarce in temperate region of Southeastern America. This is the main constraint for the implementation of these organisms as bioindicators of ecological, sedimentological, palaeoecological and physical-chemical parameters in these latitudes. Consequently, the aims of this investigation are: 1) to record the seasonal variation in composition of testate amoebae community from a temperate *Sphagnum* peatland; 2) to analyze the ecological meaning of this variation, 3) to infer the influence of pH and temperature on the distribution of the testate amoebae and; 4) to obtain useful data for further palaeoecological studies in temperate peatlands from Chile.

Keywords: Testate amoebae. Temperate peatland. Stational variation. Región de los Lagos. Chile.

INTRODUCCIÓN

Las turberas son humedales continentales palustres cuya función principal es contribuir a la estabilización de microclimas (Dugan, 1992). Las turberas se clasifican en

ombrógenas cuando el aporte hidrológico proviene exclusivamente de agua de lluvia y en geógenas cuando este aporte proviene de escorrentía superficial y/o de aguas

subterráneas (Bridgham *et al.*, 1996). Adicionalmente, de acuerdo al vegetal formador de la turba, Gignac & Vitt (1994) reconocen una categoría esfagnosa dominada por el musgo *Sphagnum* ("pon-pon").

Las turberas activas (mires) no son hábitats óptimos para muchos grupos de plantas y animales debido principalmente a la sobresaturación de agua y bajos niveles de nutrientes; sin embargo, las amebas testadas (tecamebas) son una excepción, ya que son abundantes en la película de agua de tallos y hojas de turberas esfagnosas (Heal, 1962; Corbet, 1973; Meisterfeld, 1977, 1979; Mitchell *et al.*, 1999), constituyendo uno de los mayores componentes de la microfauna de turberas. Su riqueza y distribución está regulada principalmente por el contenido de humedad, pH, temperatura, oxígeno, luz, alimento y composición de la turba (Jauhiainen, 2002).

Las amebas testadas son un grupo funcional polifilético que considera las amebas cuya célula está protegida por un caparazón o testa con una abertura oral (pseudostoma) a través de la que pueden proyectar pseudópodos que utilizan para alimentarse y moverse. Frente a condiciones abióticas adversas forman quistes de reposo o resistencia, los que quedan en un estado de latencia a la espera de condiciones favorables. Filogenéticamente se dividen en amebas testadas con pseudópodos de tipo lobópodo y de tipo filópodo (Cavalier-Smith, 2004). Las primeras pertenecen a Amoebozoa (Nikolaev *et al.*, 2005) e incluye especies grandes (>100 µm), principalmente aquellas pertenecientes a las familias Arcellidae, Centropyxidae, Diffugiidae e Hyalospheniidae (Smith *et al.*, 2008). Varios representantes de Centropyxidae y Diffugiidae tienen caparazones formados por el aglutinamiento de partículas minerales extraídas desde el ambiente. Por el contrario, varios representantes de Nebelidae tienen caparazones compuestos de idiosomas adquiridos del consumo de euglífidos (*Euglypha* spp.) como presa (Meisterfeld,

2002). En contraste, las amebas testadas con pseudópodos de tipo filópodo están estrechamente relacionadas a Foraminifera y Cercozoa (Longet *et al.*, 2004) e incluye las familias Euglyphidae y Trinematidae, las cuales contienen especies pequeñas (<100 µm) que construyen su caparazón mediante la biosintetización de idiosomas (Smith *et al.*, 2008).

Varias especies de amebas testadas tienen preferencias ecológicas bien definidas y sus caparazones se conservan bien en las turberas y otros ambientes, por lo que han sido ampliamente utilizadas como bioindicadores paleoclimatológicos, principalmente en la reconstrucción de las condiciones ambientales y climáticas sucedidas durante el Cuaternario (Meisterfeld, 1977; Barber, 1981; Tolonen, 1986; Warner, 1987; Charman & Warner, 1997; Kumar & Dalby, 1998; Mitchell *et al.*, 1999; Booth, 2002).

En Chile, los trabajos realizados sobre este grupo de organismos se iniciaron con Ehrenberg (1843), siguiéndole posteriormente los de Certes (1889), Wailes (1913), Jung (1942), Hoogenraad & De Groot (1955), Bonnet (1966), Gracia & Gadea, 1982a, b; Zapata & Rudolph (1986), Zapata & Matamala (1987), Zapata & Crespo (1990), Golemansky & Todorov (1996), Zapata *et al.* (2002), Zapata (2005), Zapata *et al.* (2007a, b), Zapata & Fernández (2008), Zapata *et al.* (2008), Fernández & Zapata (2010), Fernández & Zapata (2011) los que son principalmente de índole sistemático y han sido desarrollados, en general, en una escala temporal muy breve, correspondiendo la mayoría a estudios con sólo un muestreo en el tiempo. Por lo mismo, existe desconocimiento respecto a las variaciones estacionales que presenta la tecamebofauna y como influyen los factores abióticos sobre su distribución en la región temperada del sudeste americano. Esta situación ha limitado la aplicación de estos organismos como bioindicadores de parámetros físico-químicos, ecológicos, sedimentológicos y paleoecológicos en este rango latitudinal.

En consecuencia, este trabajo tiene por finalidad: 1) determinar en una escala temporal anual la variación en la composición de la comunidad de amebas testadas de una turbera esfagnosa temperada; 2) analizar el significado ecológico de esta variación; 3) inferir la influencia del pH y la temperatura en la distribución de las tecamebas y 4) entregar antecedentes para posteriores estudios paleoecológicos en las turberas temperadas de Chile.

MATERIALES Y METODOS

El sitio de estudio corresponde a una turbera geógena de aproximadamente 1.8 ha, ubicada en la localidad de Rucapihuel (40°34'42.5" S; 73°34'31.4" W), a 50 msnm, en la Cordillera de la Costa, provincia de Osorno, Chile (Fig. 1). En esta turbera, dominada por el musgo *Sphagnum*, existe una comunidad vegetal compuesta de chauras (*Pernettya insana* [Mol.] Gunckel y *P. mucronata* Gaudich. ex G. Don), yelmo chico (*Griselinia jodinifolia* Taub.), costilla de vaca (*Blechnum chilense* [Kaulf.] Mett.), tepú (*Tepualia stipularis* Griseb.) y ciprés de las Guaytecas (*Pilgerodendron uviferum* Florin) (Zapata, 2005). La turbera está anexa a pequeñas cuencas endorreicas de origen holocénico y se ha formado por la acumulación paulatina de materia orgánica en descomposición en un ambiente privado de aire y altamente saturado de agua todo el año (Rudolph & Crandall, 2007).

Para desarrollar este estudio se extrajo, en cada estación del año (entre agosto del 2006 hasta mayo del 2007) una muestra de turba más dos submuestras, lo que corresponde a un procedimiento de pseudoreplicación. Este último procedimiento se concibió para evitar la sub-representatividad u omisión de alguna especie, de manera de entregar una representación completa de la comunidad moderna, lo que facilitará futuras comparaciones con el registro fósil. Cada

muestra principal y sus respectivas submuestras fueron depositadas juntas en una bolsa rotulada, mezcladas y fijadas con etanol al 70 %. Paralelamente, se midió *in situ* el pH y la temperatura de la muestra utilizando un Sensor check Hanna HI 991003. Los valores entregados en los resultados corresponden al promedio obtenido de tres mediciones realizadas en cada estación del año.

Para extraer las amebas testadas se hirvieron, durante 5 minutos, 50 cm³ de turba con agua destilada, revolviendo ocasionalmente para disgregar el musgo. Posteriormente, cada muestra fue cernida primero en un tamiz de 500 µm (para eliminar el musgo y otras partículas grandes) y luego a través de un tamiz de 35 µm. El filtrado final fue centrifugado a 2000 rpm por alrededor de dos minutos para concentrar los organismos. El procesamiento en laboratorio se efectuó según lo indicado por Warner (1987) y la determinación taxonómica según los trabajos de Leidy (1879), Loeblich & Tappan (1964), Vucetich (1972a, b; 1973), Corbet (1973), Mediolli & Scott (1983), Warner (1987), Kumar & Dalby (1998), Patterson & Kumar (2002 a, b) y Zapata (2005). Las especies no fueron fotografiadas al Microscopio de Barrido, ya que son ilustradas previamente en trabajos de Zapata (2005), Zapata *et al.* (2007a, b), Fernández & Zapata (2010) y Fernández & Zapata (2011).

Para evaluar la variación estacional en la composición de la comunidad de amebas testadas en la turbera, se realizó un análisis de similitud empleando el índice de Sørensen (Sørensen, 1948; Vincke *et al.*, 2004); según el cual $S = (2C/A+B+2C)*100$, donde A y B son el número de especies registradas exclusivamente en cada una de las estaciones del año y C el número de especies comunes entre las estaciones. Con los resultados obtenidos se construyó una matriz de datos y un dendrograma mediante la técnica del ligamiento promedio y la media aritmética no ponderada (Crisci & López, 1983).

RESULTADOS

La temperatura anual de las muestras fluctuó entre 12,1 y 19,1 °C y el pH entre 5,2 y 6,1. En toda la amplitud temporal del estudio se registró un total de 60 especies (morfoespecies) distribuidas en 19 géneros y 13 familias. Algunos de estos taxa fueron registrados exclusivamente en una oportunidad, otros fragmentariamente en el tiempo y otros todo el año (Tabla I). Las estaciones de otoño, verano e invierno presentaron una riqueza específica relativamente alta con 38, 33 y 50 especies respectivamente, sin embargo, durante la primavera el número de especies decreció hallándose sólo 15 especies (Fig. 2).

Entre los géneros que dominaron en la turbera durante una o algunas estaciones estuvieron *Diffugia* (otoño, verano e invierno) y *Pontigulasia* (primavera), no obstante,

Centropyxis es el único género que está entre los taxa dominantes durante las 4 estaciones del año (Fig. 3).

El análisis de similitud confirmó la alta variabilidad estacional en la composición de la comunidad de amebas testadas, lo que fue consistente con los registros fragmentados de algunos taxa. La Tabla II corresponde a la matriz que resume las especies en común y el porcentaje de similitud entre las distintas estaciones del año. Estos valores estuvieron dados principalmente por las especies pertenecientes a los géneros dominantes y fueron graficados en un dendrograma que permite la identificación de tres clústeres utilizando un porcentaje de afinidad $\geq 50\%$ (Fig. 4). Las estaciones que tuvieron la mayor similitud respecto a la composición de taxa fueron invierno y verano (77 %). En cambio, entre las estaciones de invierno y primavera fue cuando existió la menor similitud (43 %).

TABLA I: Parámetros físico-químicos, familias, géneros, especies y distribución cualitativa de las amebas testadas registradas en la turbera de Rucapihue a lo largo del estudio. Ot: otoño; Pr: primavera; Ve: verano; In: invierno.

Estación del año	Ot	Pr	Ve	In
pH	6,1	5,8	5,9	5,2
Temperatura (°C)	12,2	19,1	17,2	12,1
Arcellidae Ehrenberg, 1843				
<i>Arcella artocrea</i> Ehrenberg, 1832	X			
<i>Arcella gibbosa</i> Penard, 1890	X			
<i>Arcella vulgaris</i> Ehrenberg, 1832	X		X	X
Assulinidae Lara et al., 2007				
<i>Assulina muscorum</i> Greeff, 1888	X			
<i>Assulina seminulum</i> (Ehrenberg, 1848)	X			
Centropyxidae Jung, 1942				
<i>Centropyxis aculeata</i> (Ehrenberg, 1832)	X		X	X
<i>Centropyxis aerophila</i> Deflandre, 1929	X	X	X	X
<i>Centropyxis arcelloides</i> Penard, 1902	X			
<i>Centropyxis constricta</i> (Ehrenberg, 1834)	X	X	X	X
<i>Centropyxis discoides</i> Ehrenberg, 1832	X		X	X
<i>Centropyxis ecomis</i> Ehrenberg, 1841	X	X	X	X
<i>Centropyxis elongata</i> (Penard, 1890)	X		X	X
<i>Centropyxis hirsuta</i> Deflandre, 1929	X		X	X
<i>Centropyxis sylvatica</i> (Deflandre, 1929)	X		X	X
Cyphoderiidae de Saedeleer, 1934				
<i>Cyphoderia ampulla</i> (Ehrenberg, 1840)	X		X	
Diffugiidae Wallich, 1864				
<i>Diffugia acuminata</i> Ehrenberg, 1838				X
<i>Diffugia bacillifera</i> Penard, 1890				X
<i>Diffugia glans</i> Penard, 1902			X	X

<i>Diffugia globulosa</i> Dujardin, 1837	X		X	X
<i>Diffugia golemansky</i> Todorov, 2005	X			
<i>Diffugia inflata</i> Penard, 1899				X
<i>Diffugia lacustris</i> (Penard, 1899)	X	X	X	X
<i>Diffugia lebes</i> Penard, 1899				X
<i>Diffugia oblonga</i> Ehrenberg, 1838	X		X	X
<i>Diffugia opulenta</i> Vucetich, 1973	X			X
<i>Diffugia penardi</i> Hopkinson, 1909			X	X
<i>Diffugia regularis</i> Vucetich, 1973	X			
<i>Diffugia vancouveri</i> Boltovskoy & Lena, 1978				X
<i>Lagenodiffugia vas</i> (Leidy, 1874)			X	X
<i>Pontigulasia bryophila</i> Penard, 1902	X	X	X	X
<i>Pontigulasia compressoidea</i> Jung, 1942	X	X	X	X
<i>Pontigulasia contusa</i> Jung, 1942	X	X		X
Euglyphidae Wallich, 1864				
<i>Euglypha ciliata</i> (Ehrenberg, 1838)			X	X
<i>Euglypha filifera</i> Penard, 1890	X		X	X
<i>Euglypha laevis</i> Perty, 1852				X
<i>Euglypha mucronata</i> Leidy, 1878				X
<i>Euglypha strigosa</i> Ehrenberg, 1872			X	X
<i>Euglypha tuberculata</i> Dujardin, 1841				X
Heleoperidae Jung, 1942				
<i>Heleopera amplicolla</i> Jung, 1942				X
<i>Heleopera petricola</i> Leidy, 1879	X		X	X
<i>Heleopera rectangularis</i> Bonnet, 1966				X
<i>Heleopera sphagni</i> (Leidy, 1874)				X
Hyalospheniidae Schultze, 1877				
<i>Hyalosphenia subflava</i> Cash & Hopkinson, 1904	X			
Lesquereusiidae Jung, 1942				
<i>Lesquereusia modesta</i> Rhumbler, 1896				X
Nebelidae Taranek, 1882				
<i>Alocodera cockayni</i> (Penard, 1910)	X			X
<i>Apodera vas</i> (Certes, 1889)	X	X	X	X
<i>Certesella certesi</i> (Penard, 1911)	X	X	X	X
<i>Certesella martiali</i> (Certes, 1889)	X	X		X
<i>Nebela collaris</i> (Ehrenberg, 1848)	X		X	X
<i>Nebela dentistoma</i> Penard, 1890			X	X
<i>Nebela penardiana</i> Deflandre, 1936				X
<i>Nebela vitraea</i> Penard, 1899			X	X
<i>Nebela wetekampi</i> Jung, 1942			X	X
Plagiopyxidae Bonnet & Thomas, 1960				
<i>Bullinularia indica</i> Penard, 1907	X	X	X	X
Trigonopyxidae Penard, 1912				
<i>Cyclopyxis arcelloides</i> (Penard, 1902)	X	X	X	X
<i>Ciclopyxis arenata</i> Cushman, 1930	X			X
<i>Trigonopyxis arcula</i> (Leidy, 1879)	X		X	X
<i>Trigonopyxis microstoma</i> Hoogenraad & de Groot, 1948	X	X	X	X
Trinematidae Hoogenraad & De Groot, 1940				
<i>Trinema enchelys</i> (Ehrenberg, 1838)	X	X	X	X
<i>Trinema galeata</i> (Penard, 1890)		X		

TABLA II: Matriz de afinidad (en %) entre las diferentes estaciones del año. Los números bajo la diagonal corresponden a las especies en común. Ot: otoño; Pr: primavera; Ve: verano; In: invierno.

Estación del año	Ot	Pr	Ve	In
Ot	—	54	70	67
Pr	14	—	50	43
Ve	25	12	—	77
In	29	14	32	—

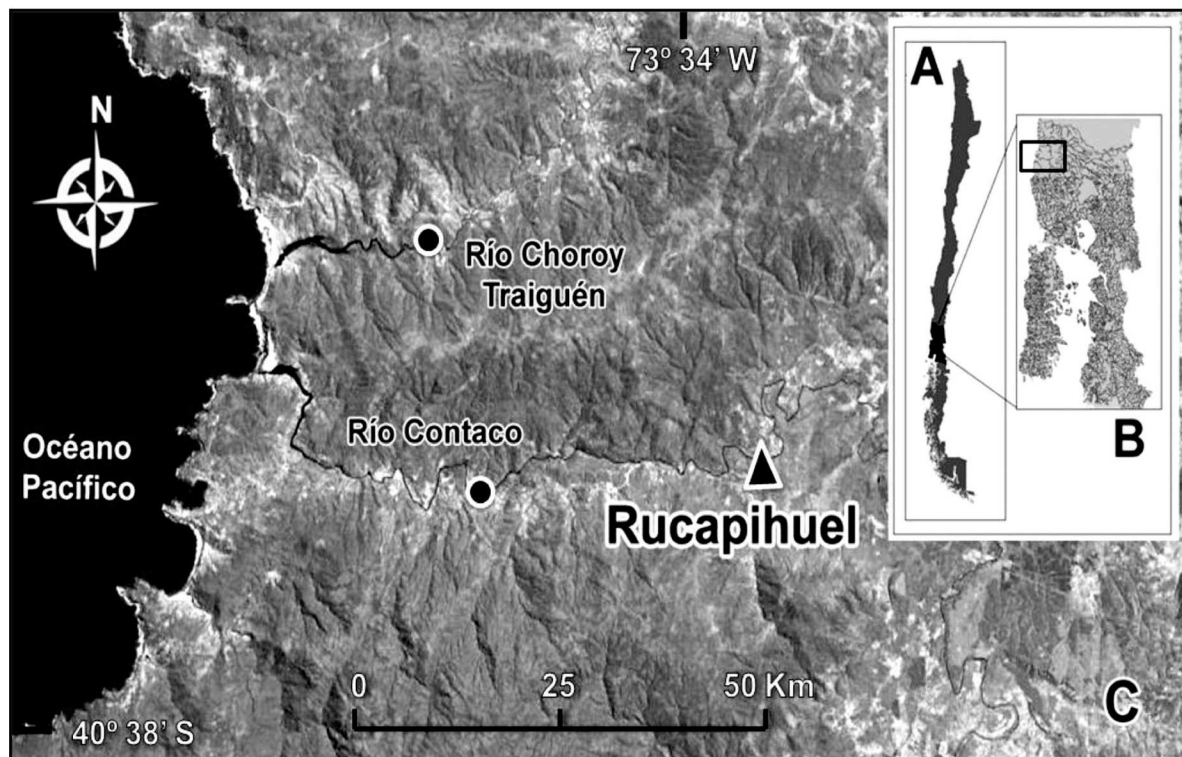


Figura 1: Sitio de estudio. A: Chile; B: Región de Los Lagos. C: Sector donde se ubica la turbera de Rucapihuel.

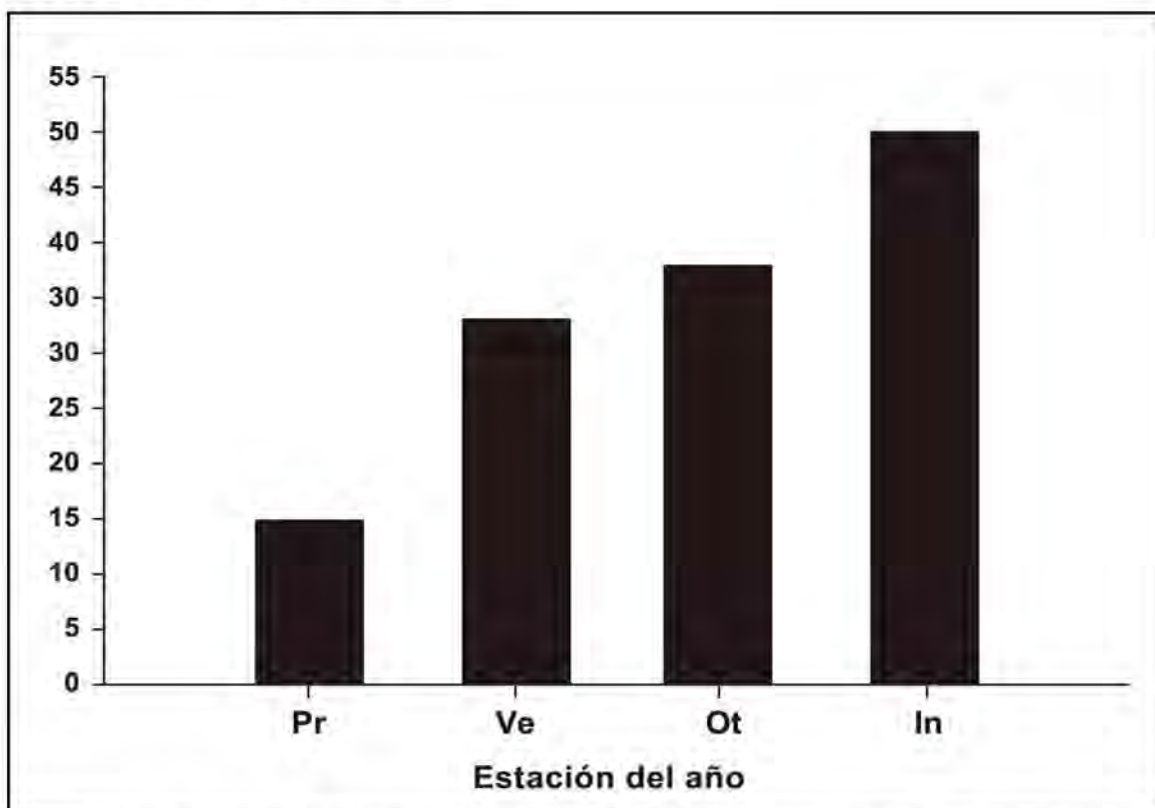


Figura 2: Número de taxa de amebas testadas registradas en cada estación del año en la turbera de Rucapihue. Pr: primavera; Ve: verano; Ot: otoño; In: invierno.

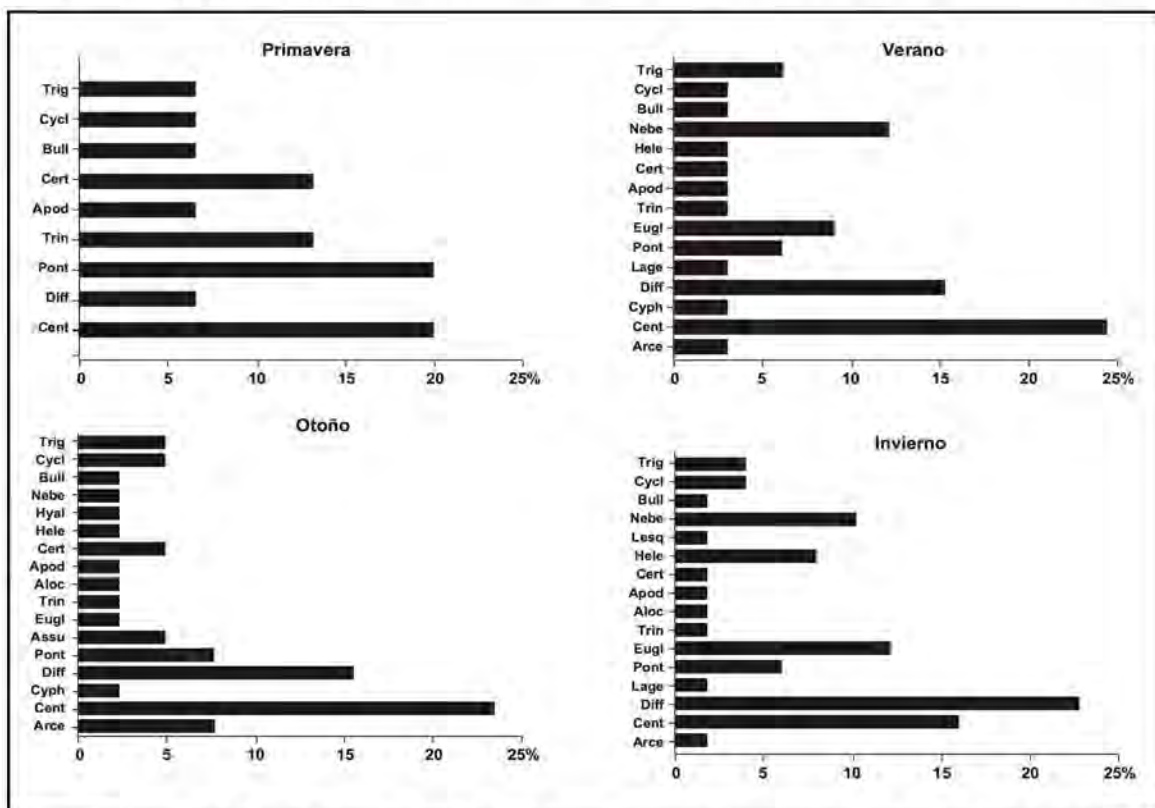


Figura 3: Porcentaje de taxa de amebas testadas pertenecientes a cada género por estación del año en la turbera de Rucapihue. Aloc: *Alocodera*; Apod: *Apodera*; Arce: *Arcella*; Assu: *Assulina*; Bull: *Bullinularia*; Cent: *Centropyxis*; Cert: *Certesella*; Cycl: *Cyclopyxis*; Cyph: *Cyphoderia*; Diff: *Diffugia*; Eugl: *Euglypha*; Hele: *Heleopera*; Hyal: *Hyalosphenia*; Pont: *Pontigulasia*; Lage: *Lagenodiffugia*; Lesq: *Lesquereusia*; Nebe: *Nebela*; Trig: *Trigonopyxis*; Trin: *Trinema*.

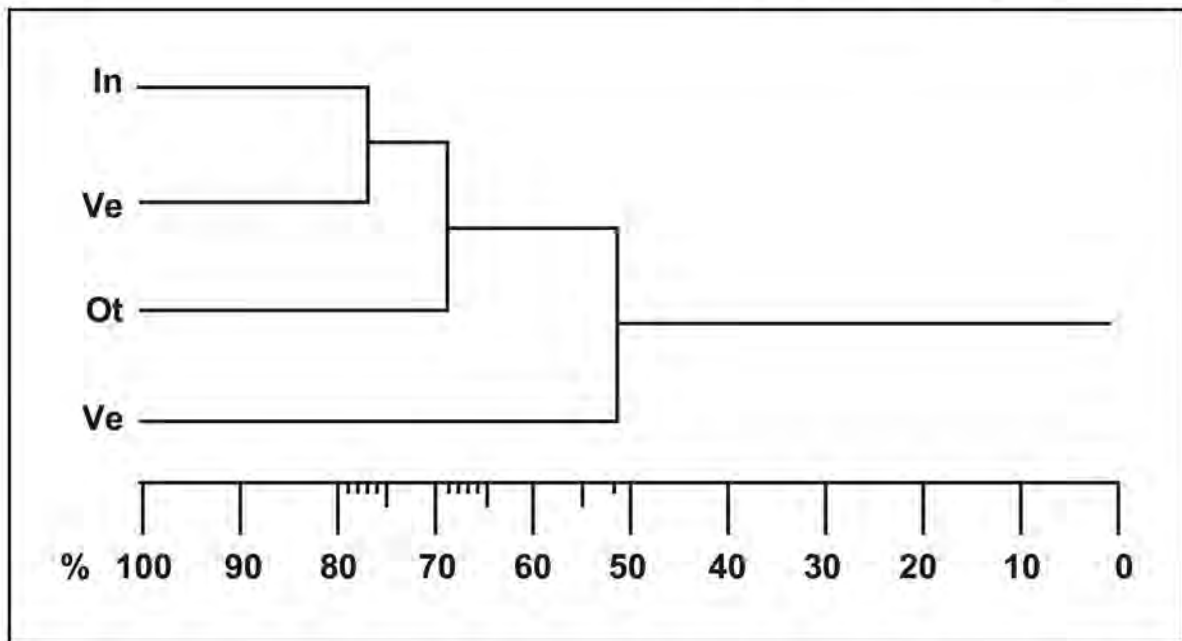


Figura 4: Dendrograma de afinidad (%) entre las estaciones del año en base a la composición de la comunidad de amebas testadas en la turbera de Rucapihue. Pr: primavera; Ve: verano; Ot: otoño; In: invierno.

DISCUSION Y CONCLUSIONES

Considerando toda la comunidad de amebas testadas registrada durante el desarrollo de este estudio podemos indicar que, en general, la turbera de Rucapihue presenta una riqueza específica similar a la reportada en turberas templadas con nulo o ínfimo impacto antrópico del sur de Chile. Por ejemplo, Zapata *et al.* (2008) reportaron para una turbera ubicada dentro del Parque Nacional Puyehue (por lo que se presume una condición de conservación prístina) un total de 42 especies, un valor cercano e incluso a veces superado por los registros obtenidos en las distintas estaciones del año que involucró este estudio, lo que podría hacer especular que la turbera está muy bien conservada. No obstante, es complicado en este caso generar conclusiones concretas o detectar alguna anomalía en esta turbera utilizando únicamente la riqueza específica como parámetro, ya que, el trabajo citado y otros desarrollados en Chile sólo implican un muestreo en el tiempo.

Por otro lado, independiente del tiempo de duración del estudio, llama la atención que en la comunidad ubicada en el Parque Nacional

dominó el Género *Nebela* y en este estudio el Género *Centropyxis*. Adicionalmente, en este último sitio existe una distribución desigual respecto al número de especies pertenecientes a otros géneros y un fuerte dominio de sólo dos taxa en cada estación. Incluso *Nebela*, uno de los géneros más comunes y abundante en las turberas templadas del sur de Chile, está ausente durante la estación de primavera.

En este caso particular, parece ser que la identificación de las especies dominantes y un análisis de la estructuración de la comunidad de las amebas testadas entregan mejor información en relación al estado de conservación real de la turbera estudiada, permitiéndonos especular que se encuentra degradada. Esto se fundamenta en que los centropíxidos son organismos que están vinculados a condiciones ambientales subóptimas (Dalby *et al.*, 2000; Patterson & Kumar, 2000a), en que el dominio exclusivo de una o más taxa en la comunidad de amebas testadas es común en ambientes muy dañados (Patterson & Kumar, 2002) y en que la distribución desigual de especies es un patrón característico en turberas altamente

impactadas, especialmente de aquellas que están siendo drenadas (Gilbert *et al.*, 2003). Todos estos antecedentes son consistentes y reafirmados con lo observado *in situ*, siendo evidente el fuerte impacto antrópico al que está siendo sometida esta turbera (*e.g.* drenado del suelo, extracción del musgo *Sphagnum*).

Los parámetros físico-químicos también parecen ser concordantes con las variaciones observadas en la comunidad de amebas testadas. Durante las estaciones más cálidas (primavera y verano) se registró una menor riqueza específica, lo que podría imputarse, en primera instancia, a que las temperaturas muy altas afectan negativamente los tiempos de generación en estos organismos (Schönborn, 1962). No obstante, en este estudio, la temperatura no se caracterizó por tener extremos altos y bajos o una variación importante entre las estaciones del año, por lo que la disminución en el número de taxa puede atribuirse a otros motivos. Además, esta última idea es contradictoria con lo reportado por otros estudios como el de Yang *et al.* (2006), quienes encontraron una fuerte correlación entre la temperatura media del mes más cálido y la riqueza de especies en el sudoeste de China.

La razón por la que existió un número menor de taxa en las estaciones cálidas puede estar fundamentada en los resultados obtenidos por Gilbert *et al.* (2003). Estos autores encontraron que la abundancia de quistes de amebas testadas está correlacionada negativamente con la temperatura, en otras palabras, los quistes son más abundantes durante las estaciones frías (invierno, otoño) y las formas activas durante las estaciones más cálidas (primavera, verano).

En este estudio, la muestra correspondiente a la primavera fue tomada a mediados de esta estación, por ende, es muy probable que los taxa que no fueron registrados hayan estado enquistados debido a que la temperatura aún no sería la óptima, puesto que los rangos reproductivos dependen altamente de este parámetro (Lousier & Parkinson, 1984; Clarke, 2003). Algo similar pudo haber sucedido en verano, ya que las muestras fueron tomadas durante el final de esta estación. Esto hace pensar que probablemente, la comunidad de

amebas testadas se mantiene relativamente estable en su composición todo el año, pero siempre con un dominio de centropíxidos debido a la mala calidad de las condiciones ambientales.

Otro parámetro significativo en la estructuración de la comunidad de amebas testadas es el pH (Patterson & Kumar, 2002 a, b; Lamentowicz & Mitchell, 2005). Por este motivo es posible que el rango de pH registrado y la mala calidad predominante en esta turbera hayan permitido que los centropíxidos se manifiesten como taxa dominante en todas las estaciones. Estos organismos son oportunistas generalistas, capaces de habitar en condiciones hostiles que no soportan otras amebas testadas. Estas condiciones incluyen temperaturas bajas (Collins *et al.*, 1990), niveles bajos de nutrientes, condiciones oligotróficas (Schönborn, 1984) y sitios altamente contaminados con Hg y As (Patterson *et al.*, 1996). Los únicos lugares en los que los centropíxidos no son exitosos es en áreas en las que predominan valores de pH bajo 5.5 (Kumar & Patterson, 2000; Patterson & Kumar, 2000 a, b).

Si bien, lo usual es que el pH oscile entre 3 y 6 en las turberas (Gorham *et al.*, 1984), en este estudio el rango fluctuó entre 5.2 y 6.1. Esto probablemente favoreció la presencia y el registro de diversos taxa con caparazón silíceo-biosintetizado de las familias Euglyphidae y Trinemmatidae, ya que, en ambientes más ácidos estos organismos tienden a disolverse. Contrariamente, los taxa con caparazón formado por la aglutinación de partículas minerales, como las familias Centropyxididae, Diffugiidae y Trigonopyxididae, se adaptan bien a los ambientes ácidos y, una vez muertos, sus caparazones se conservan muy bien en la turbera e incluso en el registro fósil (Wilmshurst *et al.*, 2003; Swindles & Roe, 2007).

La frecuencia fragmentada que presentan algunas especies a lo largo del año, sugiere la existencia de cambios continuos y estresantes en las condiciones abióticas de este ambiente. Aún así, el hecho de que exista una similitud alta entre estaciones antagonistas (*e.g.* verano e invierno), reafirma la idea de que los taxa

realmente no desaparecen durante las estaciones que les son subóptimas, sino, que se encuentran en estado latente (quistes), pudiendo consecuentemente pasar desapercibidas.

En un estudio desarrollado anteriormente en esta misma turbera, durante la primavera del 2004 (Zapata, 2005), se registraron 52 taxa, un número superior al registrado durante la misma estación en este estudio. La causa para esta gran diferencia numérica probablemente tenga su origen nuevamente en el momento de la estación (inicio o término) en el que se realizó el muestreo, pudiendo las variables abióticas condicionar la forma “activa” o “latente” de estos organismos. De igual forma, se hace evidente que el número de taxa registrados está directamente relacionado al tiempo o esfuerzo de muestreo, pues, en este estudio se registraron más especies que en el anterior.

Esto hace pensar que para evaluar todos los componentes de la microfauna y analizar e interpretar correctamente su variación estacional, es necesario incluir la evaluación de más parámetros físico-químicos, realizar estudios en los que la escala temporal de muestreo sea aún más breve (e.g. mensualmente), efectuar más muestras por sitio y apoyar los registros de taxa mediante cultivos que permitan detectar especies enquistadas evitando o minimizando su omisión.

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Appendix G. Environmental filtering and phylogenetic clustering correlate with the distribution patterns of cryptic microeukaryotic species in peatlands

MOLECULAR ECOLOGY

Environmental filtering and phylogenetic clustering correlate with the distribution patterns of cryptic microeukaryotic species in peatlands.

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Keywords:	Nearest Taxon Index (NTI), <i>Sphagnum</i> -dominated peatlands, Environmental filtering, Phylogenetic clustering/over-dispersion, Niche partitioning, Testate amoeba

Environmental filtering and phylogenetic clustering correlate with the distribution patterns of cryptic microeukaryotic species in peatlands

David Singer¹, Anush Kosakyan², Christophe V.W. Seppey¹, Amandine Pillonel¹, Leonardo D. Fernández^{1,3}, Diego Fontaneto⁴, Edward A.D. Mitchell^{1,5}, Enrique Lara¹

¹Laboratory of Soil Biodiversity, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, CH-2000 Neuchâtel, Switzerland

²Department of Zoology, Institute of Biosciences, University of Sao Paulo, Sao Paulo, Brazil

³Laboratorio de Ecología Evolutiva y Filoinformática, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario s/n, Casilla 160-C, Concepción, Chile

⁴National Research Council, Institute of Ecosystem Study, 28922 Verbania Pallanza, Italy

⁵Jardin Botanique de Neuchâtel, Chemin du Perthuis-du-Sault 58, CH-2000 Neuchâtel, Switzerland

Abstract

Very little is known about the diversity of protists and the factors that determine their diversity and community structure, as compared to other microbial groups, despite their ubiquity and known environmental roles. Environmental DNA studies are increasingly revealing the composition of natural protist communities in various environments. However, the mechanisms responsible for the assembly of these communities remain largely unknown, including the relative importance of resource partitioning, competition and environmental filtering and especially considering their phylogenetic context. To address these questions, we investigated the diversity of a complex of cryptic amoeba species in *Sphagnum*-dominated peatlands - the *Nebela collaris* group (Amoebozoa; Arcellinida) - across different microhabitats. We used an environmental DNA survey approach based on the mitochondrial cytochrome c oxidase 1 (COI) gene, allowing species-level resolution as well as phylogenetic

reconstruction. Community composition varied strongly across microhabitats and associated environmental gradients (nitrogen and water table depth). Species showed little overlap in their realized niche, suggesting resource partitioning rather than competition, and a strong influence of environmental filtering in community composition. Furthermore, phylogenetic clustering (NTI analysis) could be observed in the most nitrogen-poor areas, supporting phylogenetic inheritance of adaptations in the group of *Nebela guttata*.

Key words

Environmental filtering, Phylogenetic clustering/over-dispersion, Nearest Taxon Index (NTI), Niche partitioning, Protists, *Sphagnum*-dominated peatlands

36 Introduction

37 Molecular environmental diversity studies of microbial Eukaryotes have completely
38 challenged our vision of Earth's biodiversity. High throughput sequencing (HTS) of aquatic
39 and terrestrial habitats have revealed the existence of hundreds of thousands of phylotypes. A
40 recent study shows that eukaryotic diversity in the sunlit part of the ocean is dominated by
41 protists and exceeds that of Prokaryotes (de Vargas *et al.* 2015). Presently, a major challenge
42 in protist ecology is to understand the rules that shape the community composition of these
43 extremely diverse assemblages in natural systems, and notably if these differ from what is
44 known in macroscopic organisms.

45 Historically, the coexistence of many protist species in a single homogeneous environment
46 has been formulated by Hutchinson (Hutchinson 1961) as the "paradox of the plankton".
47 Several explanations have been given, most often based on chaotic oscillations of the different
48 populations, which rely on fast population growth and high mortality rates (Scheffer *et al.*
49 2003). However, not all protists are fast growers; for example in soil testate amoebae, only 19
50 generations per year have been reported for *Phryganella acropodia* in the River Saale
51 (Germany) (Schönborn 1992). Although fluctuations in testate amoebae populations can be
52 observed in temporal studies, these remain limited and do not reach an order of magnitude
53 (Gilbert *et al.* 2003). These dynamics rule out the impact of chaotic effects in shaping testate
54 amoeba community composition, and suggest rather that communities reach rapidly a state of
55 equilibrium. Indeed, a comparative study conducted at 40 years interval in a *Sphagnum*
56 peatland showed a remarkable stability when environmental conditions did not change
57 significantly (Buttler *et al.* 1996; Lamentowicz *et al.* 2010). The composition of such
58 communities can be rather seen as the classical product of deterministic forces due to both
59 biotic (organisms interactions) and abiotic (environmental filters) processes, plus random
60 drift. The relative importance of these drivers remains nevertheless to be determined in

communities of slow growing protists. In this respect, niche breadths of protists as well as the characteristics (stability and selectivity) of the ecosystem under consideration can be expected to play an important role (Weiner *et al.* 2012). Ecological tolerance, which can be considered as a good proxy for niche breadth (Futuyma & Moreno 1988) varies considerably amongst microbial eukaryotes, including with a single genus. For instance, species from genus *Dunaliella* are famous for thriving in different extreme environments (hypersaline, hyperacidic, high UV radiation (Pick 1998)), as well as under more mesophilic conditions (Pick, 1998). It has been shown in this particular group that adaptation to different environmental stresses was phylogenetically inherited (Gonzalez *et al.* 2001).

Sphagnum peatlands are characterised by stable but harsh conditions (oligotrophic, low pH, recalcitrant organic matter) (Dedysh *et al.* 2006; Steinberg *et al.* 2006), causing a strong selective pressure resulting in the existence of highly specific biotic communities. They are also characterised by high spatial heterogeneity, with chemical and physical conditions varying strongly in relation to microtopography, thus influencing vegetation (Okland *et al.* 2008). Members of the testate amoeba genus *Nebela* are especially common and diverse, and the *Nebela collaris* species complex is the most frequent Arcellinid testate amoeba taxon in *Sphagnum* dominated peatlands (Gilbert & Mitchell 2006). This complex includes several “genetic” species (Singer *et al.* 2015) but, as the taxonomy of this group is confusing, ecologists and palaeoecologists usually handle them as a single “type” referred to as *Nebela tinctoria* (Charman *et al.* 2000). However, multimodal patterns of distribution observed along a moisture gradient (water table depth) in *Sphagnum* dominated peatlands (Valiranta *et al.* 2012) suggests that this group includes species with diverging ecological optima. If this were the case, the question then is how far do these organisms truly co-exist in syntopy, and if so, what are the likely mechanisms involved that make this co-existence possible?

85 In order to answer these questions, we investigated species composition of members of the
86 *Nebela collaris* s.l. complex in the whole range of microhabitats existing in peatlands of the
87 Swiss Jura Mountains. In order to avoid identification mistakes which may occur with these
88 closely-resembling species, we used a metabarcoding strategy based on the specific
89 sequencing of *N. collaris* s.l. mitochondrial COI gene from environmental DNA. For each
90 sampling point, we also measured relevant environmental variables (water table depth,
91 nitrogen and phosphorus content, pH, brightness and microtopography). We determined how
92 far community composition was correlated to the environmental variables characterising the
93 different microhabitats, and if strong niche overlap occurred between species. A significant
94 niche overlap would suggest interspecific competition rather than facilitation, given the close
95 genetic relationship between species and trait similarity (Beltran *et al.* 2012). Finally, we
96 determined taxon relatedness within the different microhabitats in order to detect if
97 adaptations to a given environment were phylogenetically inherited or not. Phylogenetic
98 inheritance would result in communities of closely related taxa (phylogenetic clustering) and
99 that are organised by environmental filters; whereas lack of phylogenetic inheritance would
100 result in communities of distantly related taxa (phylogenetic overdispersion) and that are
101 organised by intraspecific competition (Emerson & Gillespie 2008; Vamosi 2014).

102 Peatlands are stable and highly selective environments which exhibit strong spatial variation
103 due to microtopography, and subsequent drastic changes in abiotic conditions. As a
104 consequence, plant communities also present high spatial structuration (Andersen *et al.* 2011).
105 Accordingly, we expect *Nebela* species to be distributed in accordance with microtopography.
106 In addition, given the low nutrient levels (and especially Nitrogen) in *Sphagnum* peatlands,
107 we expect that Nitrogen deficiency could represent a strong environmental filter that will
108 influence the composition of communities. Secondly, if *Nebela* species is associated to
109 microtopography as we suppose, niche overlap should be minimal, suggesting low

competition. In addition, as it has been observed in other protist groups, we expect that ecological preferences should be inherited in closely-related *Nebela* lineages.

Methods

Sampling and environmental variables

The study was carried out in two Swiss peatlands from the Jura Mountains: le Cachot (47°00'N 6°39'E) and Praz-Rodet (46°33'N 06°10'E). The peatlands were selected according to their similar characteristics of altitude (approximately 1000m a.s.l.) and geomorphological setting (Lotze & Hölder 1964). We collected 21 samples containing each 30g of fresh *Sphagnum* sp. (le Cachot = 11, Praz-Rodet = 10) during the period spanning from September 2013 to April 2014 in five contrasted microhabitats present in the peatland (poor fen, bog margin, bog forest, hummock and lawn; Supp. table 1). We measured water table depth (WTD), brightness and microtopography as described in Singer (2016) at the time of sampling. We then measured total Nitrogen (%), total Phosphorus (%) and pH_{H2O} following standard protocols (Carter & Gregorich 2007) (Supp. table 2). The five microhabitats had different vegetation (Singer *et al.* 2016) and environmental characteristics (Supp. fig 1). Bog margins (former peat exploitation area currently a contact zone with an adjacent hay field) were only found in le Cachot peatland.

Molecular analyses

Testate amoeba cells were extracted from 20g of fresh *Sphagnum* and concentrated by sieving at 150µm. We extracted DNA from the <150µm. using a MoBio Power Soil® DNA Isolation kit according to the manufacturer's instruction. We amplified a fragment of the mitochondrial COI gene by using the general primer LCO (Folmer 1994) and a specific primer TINCOX (CCATTCKATAHCCHGGAAATTTTC) following the protocol recommended for the

134 amplification of *Nebela collaris* s.l. species (Kosakyan *et al.* 2013). PCR steps consisted of a
135 5 min initial denaturation step in a 40 cycles program of 15 s at 95°C, 15 s at 43°C, and 1 min
136 and 30 s at 72°C with the final extension at 72°C for 10 min. The amplicons were cloned into
137 pCR2.1 Topo TA cloning vectors and transformed into *E. coli* TOP10' One Shots cells
138 (Invitrogen kit) according to the manufacturer's instructions. We amplified (M13f, M13R
139 primers (Kosakyan *et al.* 2015)) and sequenced enough inserts per PCR product to reach the
140 total diversity of *Nebela collaris* s.l. In order to evaluate if the total diversity was reached we
141 performed rarefaction curves with visual check of asymptotic behaviour for each site
142 independently and for the overall data set (sup Fig 2, 3) and we computed Chao estimates for
143 the overall data set using the R vegan package (V.2.3-5 (Oksanen J *et al.* 2015), Sequencing
144 was carried out using a BigDye197 Terminator Cycle Sequencing Ready Reaction Kit
145 (Applied Biosystems) and analysed with an ABI-3130XL DNA sequencer (Applied
146 Biosystems).

147 *Phylogenetic reconstructions*

148 Phylogenetic analysis was used to determine the phylogenetic position of the sequences
149 obtained. We built a reference database containing 32 COI sequences (obtained from single
150 cells) of the *Nebela collaris* species complex from GenBank (Heger *et al.* 2011; Kosakyan *et*
151 *al.* 2013; Kosakyan *et al.* 2012; Singer *et al.* 2015) plus three sequences of *Nebela tubulosa*
152 (GenBank accession number: JN849020, JN849021, JN849061) used as outgroup. Sequences
153 were aligned using the Clustal W algorithm (Thompson *et al.* 1994) (Supp. PDF 1). COI gene
154 sequences of the *Nebela collaris* species complex contained some post-transcriptional
155 mitochondrial editing resulting on the insertion of single nucleotides in the gene sequence
156 (Kosakyan, 2013), these single nucleotides were further removed for all subsequent analyses.
157 Trees were reconstructed using Bayesian and maximum likelihood (ML) approaches as in
158 Kosakyan *et al.*, 2013 and Singer, 2015. A Bayesian Markov Chain Monte Carlo (MCMC)

analysis was performed using MrBayes v3.2 (Ronquist *et al.* 2012) with a general time reversible model of sequence evolution. A ML tree was build using the RAxML v7.7.1 algorithm (Stamatakis *et al.* 2008) using the GTR+G+I model. Model parameters were directly estimated in RAxML.

Phylotype identification

To evaluate the number of independent evolving units within *Nebela collaris* s.l., we used the Automatic Barcode Gap Discovery (ABGD) method (Puillandre *et al.* 2012) as describe in (Fontaneto *et al.* 2015). ABGD is an automated procedure that clusters sequences into candidate species based on pairwise distances by detecting differences between intra- and inter-specific variation without any *a priori* species hypothesis (Puillandre *et al.* 2012). We used the web-server of ABGD <http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html> with the Jukes-Cantor (JC69) model and the default parameters to run this analysis.

Niche partitioning and competition

In order to convert clone numbers to relative proportions of individuals from each species, we calculated the “corrected clone number” (C_{x_corr}) of each phylotype as described in (Kosakyan *et al.* 2015). Briefly, Kosakyan *et al.* (2015) have demonstrated that there is a significant correlation between the Biovolume of a selected species and the number of clones obtained for the species within the community. C_{x_corr} is obtained by dividing the biovolume of the largest species (*Nebela collaris*) by the biovolume of the given species multiplied by the number of clones found in the community. For the phylotypes that could not be assigned to a described species, and thus could not be associated to a known bio-volume, the value of the largest species (*Nebela collaris*) was selected (Supp. table 3) in order to avoid an over-representation of the phylotype within the community.

182 We assessed if species assemblages (relative proportions of each species for each sample) of
183 the *Nebela collaris* group changed significantly among microhabitats using a redundancy
184 analysis (RDA) with water table depth, pH, nitrogen content used as explanatory variables.
185 These variables correspond to the main ecological gradients in *Sphagnum*-dominated
186 peatlands (Bridgham *et al.* 1996; Bridgham *et al.* 2001).

187 Direct experimental evidence cannot be provided to prove if competition takes place or not
188 given that *Nebela* spp. do not grow in culture; thus, we had to assess only indirect evidence to
189 evaluate if competition may take place or not. Under this scope, we evaluated this possibility
190 by computing (1) C-scores to evaluate if niche breadth is significantly different between
191 species and (2) Pianka index, to estimate whether niches overlap.

192 Species co-existence can be impossible either because they occupy different niches (reduced
193 fundamental – or grinnellian - niche overlap) or because they replace each other due to strong
194 competition (high potential niche overlap but distinct realised – eltonian - niches) (Elton
195 1927; Grinnell 1917). We tested the null hypothesis that phylogenetically close species of the
196 *Nebela collaris* group have the same niche breadth and thus, exhibit strong interspecific
197 competition (i.e. they exhibit a strong niche overlap and cannot co-occur) using the C-score
198 index (Stone & Roberts 1990). This method requires a species x site matrix to calculate the
199 species occurrence at a given site in the absence of another one. If a high number of species
200 do not co-occur, then a checkerboard pattern arises in the matrix. The occurrence of this
201 pattern is interpreted as an indication of exclusive competition between species (Diamond &
202 Cody 1975; Gotelli & Graves 1996; Stone & Roberts 1990). The statistical significance of
203 this analysis was evaluated with a row-column null model (50,000 random matrices, $P <$
204 0.05), a conservative algorithm that minimises Type I errors (Gotelli 2000). C-score values
205 higher than expected supports the null hypothesis, while values lower than expected reject the

206 null hypothesis. C-score and null model calculations were computed using the R package
207 EcoSimR (v.0.1.0) (Gotelli & Ellison 2013).

208 We calculated Pianka's niche overlap index (Pianka 1973) in order to evaluate if species
209 could be expected to compete with each other using another line of evidence. This index
210 requires an incidence matrix with species as rows and niche categories as columns to analyse
211 how much overlap do exist in the use of a given resource axis among species. Pianka's index
212 ranges from 0 (no resource used in common between two given species) to 1 (complete
213 overlap in resource use). The statistical significance of this analysis was evaluated with a row-
214 column null model (50,000 random matrices, $P < 0.05$) a conservative algorithm that
215 minimizes Type I errors (Ulrich & Gotelli 2010). Values lower than expected (below the 95%
216 confident intervals of the null model expectations) support the occurrence of low niche
217 overlap between taxa, while values higher than expected (above the 95% confident intervals
218 of the null model expectations) reject this hypothesis. Pianka's index and null model
219 calculations were conducted using EcoSimR (v.0.1.0) (Gotelli & Ellison 2013).

220 We calculated Nearest Taxonomic Index (NTI) to evaluate the phylogenetic structure of
221 species assemblages in each sample and compare the obtained values across microhabitats.
222 The NTI index is more sensitive to patterns of evenness and clustering closer to the tips of the
223 phylogeny than other index such as the Net Relatedness Index (NRI) (Kembel *et al.* 2010),
224 and the use of clearly identified fine-scale taxonomic units such as phylotypes is known to
225 improve the reliability of the method (Koeppel & Wu 2014). Studies based on microbial
226 communities suggested that a phylogenetic clustering (NTI>0) may indicate habitat filtering
227 (Horner-Devine & Bohannan 2006; Pontarp *et al.* 2012). To the opposite, phylogenetic over-
228 dispersion (NTI<0) was also detected in communities and potentially connected to
229 interspecific competition (Koeppel & Wu 2014). The NTI was calculated using Picante (V.
230 1.6-2, Kembell *et al.* 2010) within R. Analysis was run with sequences abundance information

231 and the parameters used for the phylogeny reconstruction were the same as described above.

232 The Phylogeny was pruned in keeping the sequence of the more abundant haplotype of each

233 phylotypes defines with the ABGD analysis. In order to understand the role of the

234 environmental correlates on NTI and NRI, we build linear models with NTI or NRI values for

235 each site as the response variable and a set of environmental measurements as explanatory

236 variables. Most of the explanatory variables expressed habitat features (i.e. N, P, N/P, pH,

237 Water table depth, conductivity), but we also included the potential confounding effect on

238 NTI of observed species richness, measured as number of phylotypes. Some variables (i.e.

239 water table depth and conductivity) were log transformed to improve model fit.

240 We started by trying to minimise the effect of multicollinearity by checking for correlation

241 between the explanatory variables: for each pair of continuous variables with Pearson

242 correlation parameter higher than 0.75 we removed the one with the highest correlation values

243 with the other variables. The only variables with > 0.75 correlation parameter were N and P

244 (Supp. Table 4), and we retained N for the subsequent analyses.

245 After this preliminary step, we run a full model for NTI and a full model for NRI including all

246 the remaining variables. We directly assessed model fit by: plotting the distribution of the

247 model residuals to visually check if their distribution was approximately Gaussian; plotting

248 the predicted versus fitted residuals to check for the Gauss-Markov assumptions; plotting the

249 normal Q-Q plot to compare theoretical and observed quantiles; checking whether Cook's

250 distances were all < 0.5 (Crawley 2012). Moreover, in order to assess the relative importance

251 of each predictor in the statistical models, we used a model averaging approach (Burnham &

252 Anderson 2002): the set of sub-models including all possible combinations of the explanatory

253 variables was generated and the Relative Importance (RI) value of each variable was

254 calculated, on a scale from 0 to 1, as the sum of the Akaike weights of the sub-models in

255 which the variable appears; better models have larger Akaike weights, and a variable that

contributes more to model fit will thus have a higher relative-importance value, closer to 1. Model averaging was performed with the R MuMIn package version 1.10.5 (Barton 2014).

Results

The cumulative curves for the overall data set and for the single sites reached asymptotic values (sup Fig. 2,3), and the observed species richness ($n=9$) is within the confidence interval of the Chao estimates (9.9 ± 2.1).

The ABGD analysis including our 1028 sequences (Supp. PDF 1) and the 32 reference sequences available in GenBank for the *Nebela collaris* s.l. group identified a distinctive “barcode gap” centred around 7% of divergence of the COI sequences and delimited 14 genetic clusters as candidate species (Fig 1). This result was consistent in all recursive partitions with prior intraspecific genetic divergence values between 0.28% and 0.77%, and we considered it more likely than the two other alternatives (defining 54 candidate species with intraspecific divergence values below 0.2% or considering only two species with an intraspecific divergence values higher than 1.29%).

According to the most conservative estimate in ABGD 14 phylotypes could be identified. Nine of the phylotypes were present in our samples, whereas four other were represented only by sequences from GenBank (Fig. 1). Six of the phylotypes in our samples corresponded to known species (*N. collaris*, *N. guttata*, *N. tincta*, *N. rotunda*, *N. pechorensis* and *N. gimlii*) and the three other (referred to here as unknown phylotypes UP 1-3) represented probably undescribed species (Fig 2). UP3 corresponded to a sequence derived from a single cell documented in previous studies (Kosakyan *et al.* 2013; Kosakyan *et al.* 2012) (Fig. 1). The four more abundant phylotypes could be affiliated to the species *N. collaris*, *N. guttata*, *N. tincta* and *N. gimlii*. The less represented phylotypes, which never exceeded 20% of the total sequences within individual samples, were found mostly in the peatland margins (Fig 2).

280 Numbers of COI sequences found for each phylotype are presented in the Supp. table 1.
281 Obtained sequences were deposited in GenBank under the following accession numbers: (to
282 be provided after acceptance of the manuscript).

283 The RDA analysis (Fig 3) of *Nebela collaris* s.l. communities in the different microhabitats
284 from the two peatlands showed that species assemblages did not differ between the two
285 peatlands ($p = 0.093$) and that the effect of sampling date was not significant ($p = 0.08$), but
286 differed significantly among microhabitats ($p = 0.018$) with only limited overlap between
287 lawns and hummocks in the first two axes of the ordination space (Fig. 3). The first two axes
288 together explained 59.6% of the total variance, with two variables, N (total nitrogen content)
289 and WTD (water table depth) explaining respectively 24% and 10% ($p=0.003$ and 0.037 ,
290 respectively).

291 The C-score index was significantly lower than expected (C-score Observed = 0.3611;
292 expected = 5.5373, $\pm$ SD 0.078; $p = 0.01$), thus suggesting an absence of competitive
293 exclusion between the different species of genus *Nebela* (i.e. they co-occur in the same site
294 more often than expected). Furthermore, the Pianka's index was lower than expected by
295 chance (Pianka's index; Observed = 0.369, expected = 0.773, $\pm$ SD 0.00194, $p < 0.0001$),
296 suggesting that overall *Nebela* species exhibit a low niche overlap and that therefore, they
297 perform an effective partitioning along the resource axes measured (habitat breadth). Taken
298 together, these two analyses suggest that competition does not play an important role in
299 shaping the assemblages of *Nebela* sp. species in the surveyed sites.

300 The regression coefficient estimated in the full linear model including (estimate, Standard
301 error and p-value) together with the RI value obtain with the model averaging approach
302 revealed N content as the most relevant environmental correlate for NTI (sup table 5) and for
303 NRI (Supp. Table 6). The NTI values were closer to 0 (i.e. neither phylogenetic clustering nor
304 over-dispersion) in microhabitats where the N content was high (e.g. fens), while NTI values

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303 NRI (Supp. Table 6). The NTI values were closer to 0 (i.e. neither phylogenetic clustering nor
304 over-dispersion) in microhabitats where the N content was high (e.g. fens), while NTI values

329 species complex is likely much higher and more species are likely to be found in other
330 habitats and/or regions of the World.

331 The present study revealed that the different *Nebela* spp. were not randomly distributed in the
332 studied peatlands, but were strongly correlated to microhabitats, thus following the
333 microtopology present in the different microhabitat. Vascular plant and bryophyte
334 communities also follow this trend, and are at least partly responsible for changes in the local
335 physicochemical conditions. Especially, *Sphagnum* spp. is well known to strongly influence
336 key factors such as pH and ion content of their direct environment called bryosphere (Clymo
337 1973; Lindo & Gonzalez 2010; Van Breemen 1995). Testate amoebae species distribution
338 may be influenced by plants directly (e.g. through facilitation or allelopathy) or indirectly
339 (e.g. through environmental modifications induced by the plants (Imparato *et al.* 2016;
340 Philippot *et al.* 2013)), but the possible existence of such mechanisms remains to be
341 determined. Irrespective of plant community composition, the distribution of *Nebela* spp. was
342 highly correlated with N content (Fig 3, 4). Nutrient deficiency is a common trait in
343 *Sphagnum*-dominated peatlands and especially in the hummock microhabitats (Bridgham *et al.*
344 1996). This lack of nutrients is known to shape prokaryotic communities, and peatlands
345 have been cited as an example of extreme environment for this particular reason (Dedysh *et al.*
346 2006). It is, thus, likely that N deficiency acts as an environmental filter which influences
347 species composition in *Nebela* communities. Other abiotic factors commonly measured in
348 peatlands, like depth to water table, pH and P content were not correlated with community
349 composition. A likely reason is that the range of variation in the microsites where *Nebela*
350 species were found was too narrow to exert a detectable pressure on the communities.
351 Altogether, these findings suggest that environmental filters do influence *Nebela* species
352 distribution differently supporting the hypothesis that species differ in their respective
353 ecological preferences.

Phylogenetic niche conservatism (Losos 2008; Wiens *et al.* 2010) predicts that two closely-related species sharing the same niche should exclude each other. In our case, C-score values suggested a low likelihood that the presence or absence of one species in a given site was directly affected by the presence of other species. Likewise, the Pianka index suggested that, overall, species of *Nebela* exhibit low niche overlap, and that species perform an effective partitioning along the resource axis measured (habitat breadth). However, these assumptions are solely based on co-occurrence of species on the same sites and thus cannot be considered as strong evidence against species competition. Observations based on species traits can nevertheless corroborate this hypothesis. Indeed, *Nebela* species can be differentiated, amongst other features, based on the width of the shell aperture and shell size/biovolume (Kosakyan *et al.*, 2013). These morphological traits have been correlated to prey size, larger species with broader apertures feeding on large preys (Jassey *et al.* 2014). In this study, *N. tincta* and *N. guttata* have been found very often in the same sample (Fig. 2), and because *N. tincta* has a aperture on average 19% wider than *N. guttata* and a biovolume 44% larger, it can reasonably be expected that the two species have different food regimes (Kosakyan *et al.*, 2013, 2015). These two species would therefore be expected not to compete, or at least not for preys, and thus be able to co-occur due to resource partitioning.

Furthermore, we also demonstrate that phylogenetic clustering occurs in the most N-depleted areas of the peatland. This is illustrated by strongly positive NTI values in microhabitats where N concentrations were lowest (hummocks and lawns), meaning that organisms encountered in these microhabitats are phylogenetically more closely related than expected by chance (Webb *et al.* 2002). Typical species from N-poor environments are *N. guttata* and *N. tincta*, which are phylogenetically closely related (Fig 1). Therefore, it can be hypothesized that the most recent common ancestor of *N. tincta* and *N. guttata* underwent particular adaptations to exceptionally N-poor environments. These adaptations might be slow

379 population growth and low rates of metabolism. In contrast, in microhabitats where N is less
380 limiting (i.e. forest, fen and peatland margins), NTI values were close to 0, which means that
381 communities tend to have a more heterogeneous phylogenetic composition. When NTI values
382 are plotted against N content, this relationship appears even more clearly (Fig 4). We interpret
383 this situation as a decrease in selective pressure in richer (or more mesophilic) environments,
384 allowing higher numbers of species, not phylogenetically related, to co-occur.

385 Here, we show that the different species, as defined by COI (Fig. 2) have contrasted
386 distributions among microhabitats within *Sphagnum* peatlands (Fig. 1-2), while each
387 microhabitat type harboured very similar communities, independently of the studied peatland
388 (Fig. 3). Therefore, species distribution is not random across microhabitats and community
389 patterns are indeed correlated to the main ecological gradients characterising *Sphagnum*
390 peatland (Fig. 3).

391 These species also have different niche breadths and, where they co-occur, are more likely to
392 be affected by resource partitioning than competition. Finally, our results suggest that niche
393 conservatism takes place in genus *Nebela*, where only a few species can adapt to excessively
394 low N contents. Altogether, we show that habitat selection and resource partitioning are
395 effective in shaping *Nebela* communities, and might be possible drivers for diversification in
396 the genus *Nebela* and other “slow-growing” protists in general.

397

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Fig. 1) Bayesian Markov Chain Monte Carlo consensus tree of 32 single cells of *Nebela collaris* s.l. mitochondrial cytochrome oxidase, subunit I (COI) sequences and 1028 environmental sequences, plus 3 sequences of *Longinebela tubulosa* as outgroup. The numbers along the branches represent, respectively, bootstraps obtained by maximum likelihood method and posterior probabilities as calculated with Bayesian analyses. Only values above 50/0.50 are shown. Sample colours indicate the five different microhabitats (red = lawn, yellow = forest, blue = hummock, orange = margin, black = fen).

Fig. 2) Communities profiles of testate amoebae from the *Nebela collaris* complex s.l. extracted from *Sphagnum* samples from two peatlands in the Swiss Jura Mountains. Each barplot represents the relative ratio of the clone sequences corrected by the cell biovolume (Kosakyan, 2015). UP = Unknown Phylotype.

Fig. 3) Redundancy analysis of *Nebela collaris* s.l. communities extracted from *Sphagnum* sample collected in Le Cachot bog (empty symbols) and Praz-Rodet bog (filled symbols). Environmental variables ($p < 0.05$) are represented by the arrows.

Fig. 4) NTI values in function of Nitrogen content (%). Linear regression (p -value = 0.022), dotted lines correspond to the 95% confidence interval.

Supp. table 1) List of sampling location of testate amoebae from the *Nebela collaris* complex extracted from *Sphagnum* samples from two peatlands in the Swiss Jura Mountains. Number of clones, and GenBank numbers corresponding to each phylotype are presented for each sample.

Supp. table 2) Environmental variables. Brightness (classes: 0=0-25%, 1=25-50%, 2=50-75%, 3=75-100% of brightness). Microtopography (classes: 0=0-5cm, 1=5-10cm, 2=>10cm).

Supp. table 3: Biovolume correction factors (Kosakyan 2015, Singer 2015), the Unknown Phylotype (UP) were assigned a value of 1.

Supp. Table 4) Matrix of Pearson correlations established between 6 quantitative values of the study (N, P, N/P, pH, Water table depth and Conductivity)

Supp. table 5) Matrix of regression coefficient (estimate, standard error, and p value) of the linear model of the NRI value versus N, P, N/P pH, water table depth and conductivity. Together with the relative importance of each variable found with the model averaging approach (Bartón 2014)

Supp. table 6) Matrix of regression coefficient (estimate, standard error, and p value) of the linear model of the NTI value versus N, P, N/P pH, water table depth and conductivity. Together with the relative importance of each variable found with the model averaging approach (Bartón 2014)

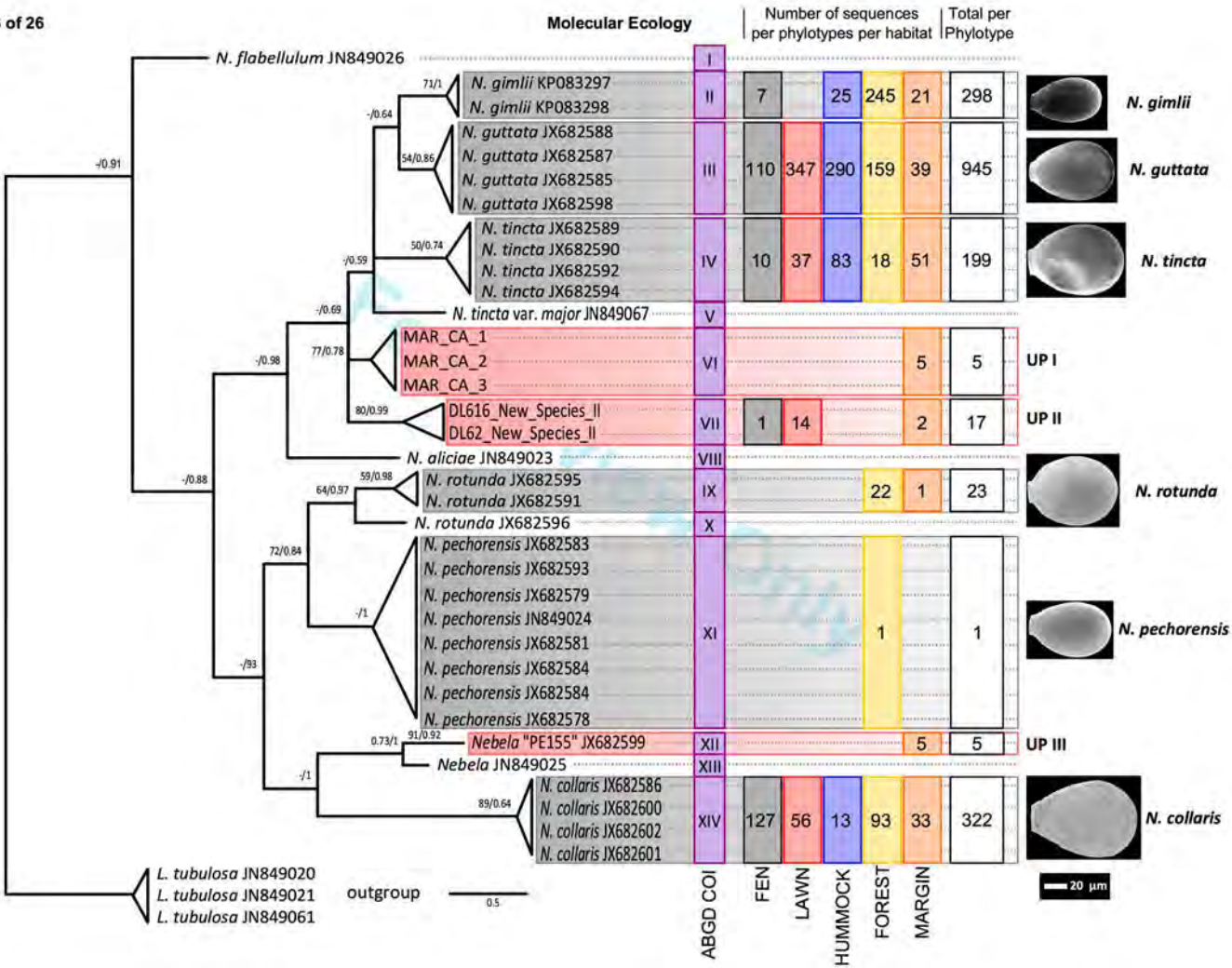
Supp. fig 1) Hierarchical clustering result of the samples according to scaled environmental variables (pH, water table depth, N content, brightness and micro-topography) computed with Euclidean distances and complete linkage method.

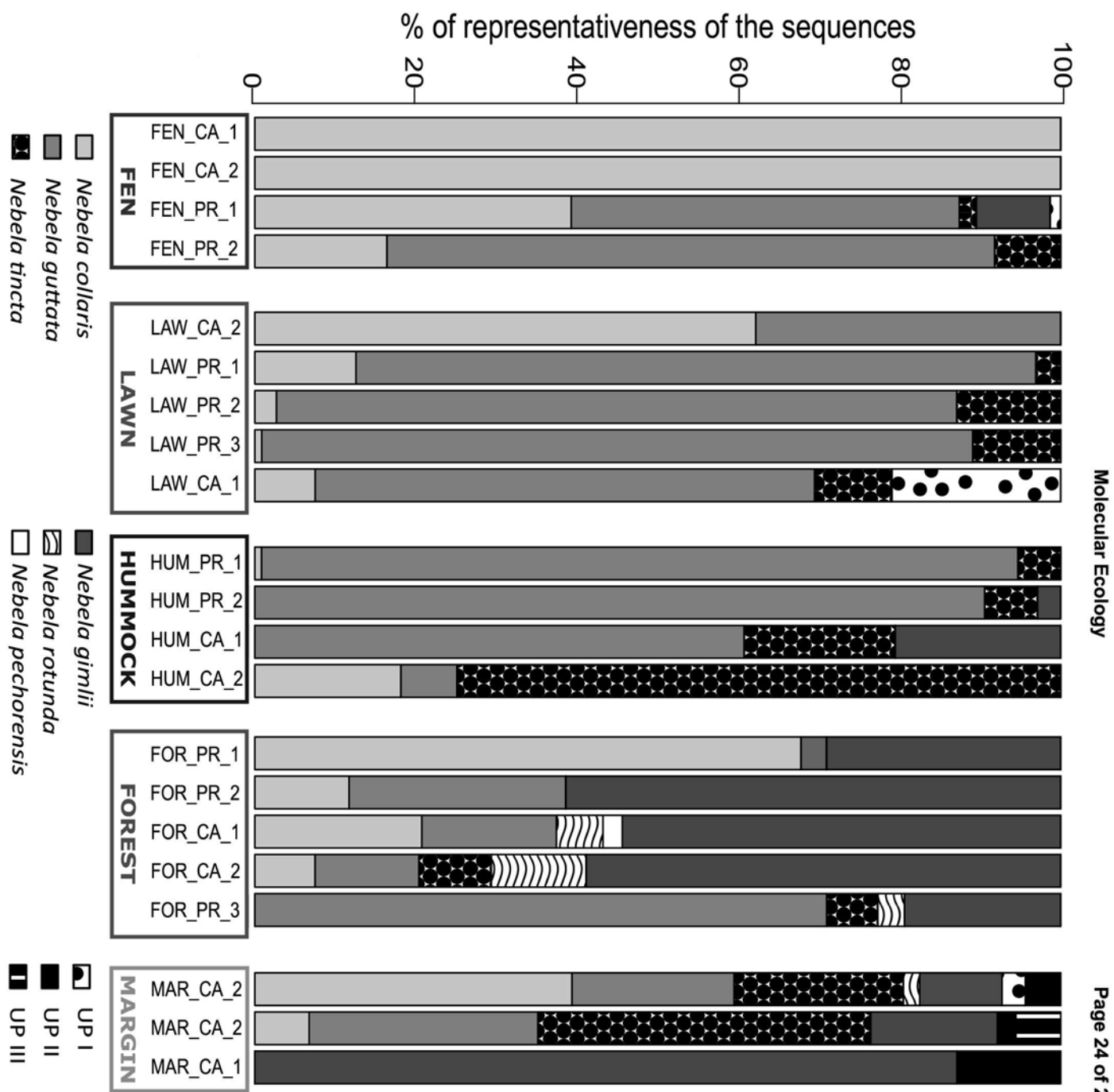
Supp. fig 2) Rarefaction curves of each sample with the number of sequences obtained after biovolume correction. HUM = hummock (blue), LAW = lawn (red), FEN = fen (black), MAR= margin (orange), FOR = forest (yellow), CA = Cahot, PR = Praz-Rodet.

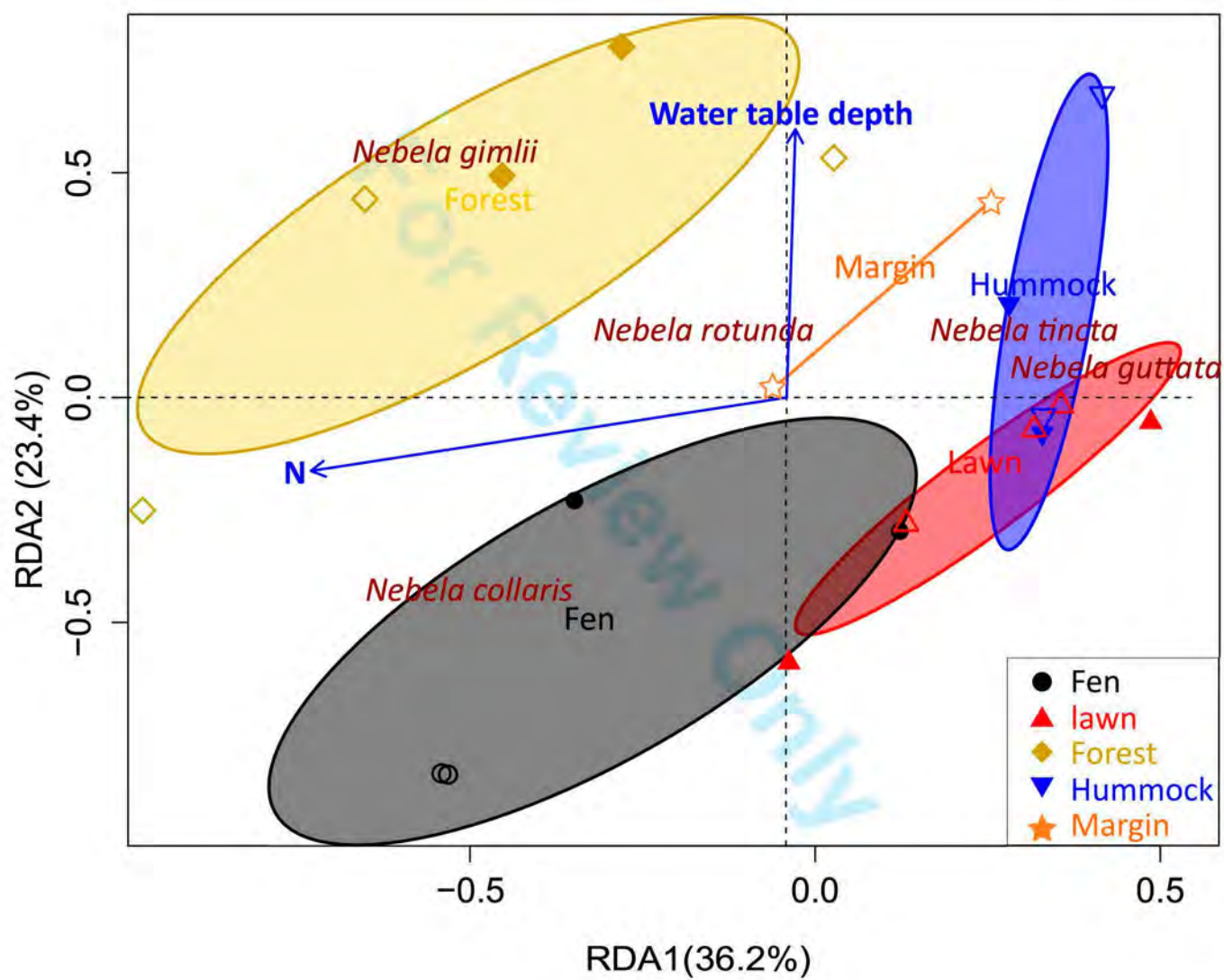
581 Supp. fig 3) Rarefaction curve of the overall dataset with the number of sequences obtained
582 after biovolume correction.

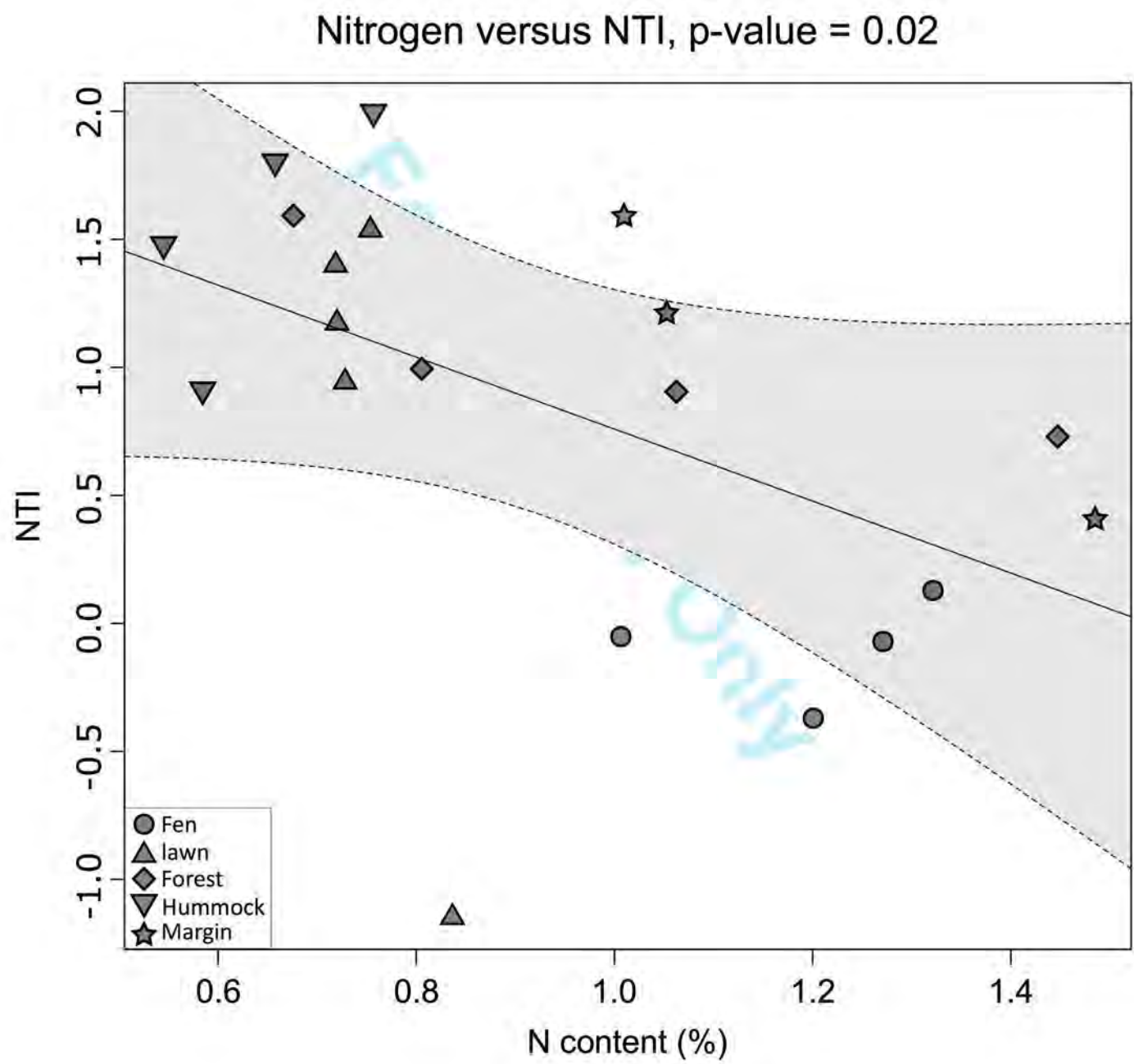
583 Supp. PDF 1) Alignment of the *Nebela collaris* s.l. sequences and the reference database used
584 for building the tree.

For Review Only









Appendix H. Acknowledgments

This PhD thesis is dedicated to my wife Fabiola and our coming baby girl, Matilda. Fabiola, I could never have finished this PhD without you and your love. Thank you!

I would like to thank my supervisors: Edward Mitchell, Enrique Lara and Cristián Hernández. Edward: thank you for your constant support and for acting as my scientific “Maecenas” (or patron) during my stay in Switzerland. Thank you also for your friendship! I spent really good times with you, especially during our field trips to Spain and Chile. Enrique: I thank you for all the feedback you gave me during my PhD. I will also never forget all the good times we shared together in the lab! You are a great friend. Cristián: Thank you for accepting me in your lab. Undoubtedly, that symbolic act represented the beginning of my professional growth.

I would also like to thank the support and friendship of all my colleagues of the Universidad de Concepción (Chile) and the University of Neuchâtel. Special thanks to (in alphabetic order): Aldo Arriagada, Alfonso Rojas, Alejandra Panzera, Amandine Pillonel, Angélica Serrano, Anush Kosakyan, Benjamín Guzmán, Bertrand Fournier, Carlos Zamora, Carmen Fuentealba, Christian Muñoz, Cristian Canales, Christophe Seppey, Clément Duckert, Dalinda Bouraoui, David Singer, Delphine Devenoges, Dylan Tatti, Franzi Sorge, Horacio Pérez, Ildikò Szelec, Isabel Koenig, Jorge Avaria, Lenka Luptakova, Margarita Ruiz de Gamboa, Matthieu Mulet, Miguel Angel Poisson, Monika Reczuga, Oriet Carvajal, Oscar Inostroza, Pastor Enmanuel Pérez, Quentin Blandenier, Reinaldo Rivera, Sandra Ferrada, Sandra Sampertegui and Yangmin Qin.

I would also like to express my deepest gratitude to Cynthia Rojas (Dirección de Postgrado, UdeC), Matilde Mella (Dpto. Zoología, UdeC), Christiane Bobillier (IDPOB, UniNe) and the secretary of the Institute of Biology (UniNe) for their constant help with all the paperwork I did during my PhD.

I also thank the constant support and good vibes of my family. From Osorno: my mother Isabel, my father Álvaro and siblings Simón, Nicolás, Álvaro, Isabel and Florencia. From Puerto Varas: Carmencita, Jessica, Gunther, Andrés, María Elena, Iván and Daniela. From Valparaíso: abuelita Elena and “toda la manada”. From Santiago: Tío Teto, Tía Maritza and Daniel.

Finally, I thank the financial support of the following institutions: (1) CONICYT – Chile (beca de doctorado nacional N° 21110037 & beca de co-tutela doctoral 78130011); (2) Programa de Doctorado en Sistemática y Biodiversidad and Dirección de Postgrado – Universidad de Concepción; (3) the Interuniversity Doctoral Program in Organismal Biology – Université de Neuchâtel; (4) the Laboratory of Soil Biodiversity – Université de Neuchâtel; and (5) the Wildlife Conservation Society (Karukinka-grant N° 2012)

Appendix I. Curriculum vitae

CURRICULUM VITAE

Leonardo D. Fernández

Laboratory of Soil Biodiversity
University of Neuchâtel
Rue Emile-Argand 11
CH-2000 Neuchâtel
Switzerland



www.researchgate.net/profile/Leonardo_Fernandez4



limnoleo@gmail.com



+41 (0)79 218 24 26

Summary

My research is focused on clarifying the processes that originate and maintain eukaryotic microbial diversity at different spatial scales. In order to achieve this goal, I use approaches that fall within the disciplines of community ecology, landscape genetics, population genetics, macroecology and biogeography. I have a vast experience on writing and submitting successful funding proposals for research projects to Chilean and international private and government funding agencies, as well as solid personal and professional connections with researchers from different Latin American and European countries. I also hold a strong publication record: I am the first author in several of my papers and almost all of them have been published in scientific journals of national and international prestige.

Languages skills

<i>Language</i>	:	<i>Reading</i>	<i>Writing</i>	<i>Listening</i>	<i>Conversation</i>
English	:	Excellent	Very Good	Very Good	Fluent
French	:	Good	Moderate	Moderate	Moderate
Spanish	:	Mother tongue			

Education

2011-2016 (joint PhD)	:	(1) PhD in Biological Sciences. University of Neuchâtel, Switzerland (2) PhD in Systematics and Biodiversity. University of Concepcion, Chile
2010	:	BSc and Master degree in Marine Biology. Universidad de Los Lagos, Chile

Awards, grants and scholarships

2016	:	<i>Young Testate Amoeba Researcher Award</i> (YoTAR/ISOP award), granted by the International Society of Protistology (ISOP) and the International Symposium on Testate Amoebae
2016	:	<i>Congress grant</i> , awarded by the University of Neuchâtel, Switzerland
2014-2015	:	<i>Research grant</i> , awarded by the Wildlife Conservation Society (WCS). Only 1 grant was awarded in South America
2014	:	<i>Mobility grant</i> , awarded by the University of Neuchâtel, Switzerland

- 2013 : *Mobility grant*, awarded by the Universidad de Concepción, Chile
- 2013-2015 : *Joint-PhD scholarship to study abroad*, awarded by CONICYT-Chile. [Only 7 scholarships were awarded in Chile](#)
- 2011-2015 : *Doctoral scholarship to study in Chile*, awarded by CONICYT-Chile
- 2011 : *Tuition-waiver scholarship*, awarded by the Universidad de Concepción
- 2005-2010 : *Full tuition-waiver scholarship*, awarded by the Universidad de Los Lagos

Employment and teaching experience

- 2017-present : Researcher / Lecturer. Research Center of the University Bernardo O'higgins (CIRENYS), Chile
- 2016 : Assistant professor (part-time). BSc Course: Ecology. University of Americas, Chile
- 2015 : Scientific collaborator at the Soil Biodiversity Laboratory. University of Neuchâtel, Switzerland
- 2015 : Contribution to teaching. BSc Course: Molecular Biology. University of Neuchâtel, Switzerland
- 2011-2013 : Contribution to teaching. BSc courses: Zoology, Neontology and Macroecology. University of Concepción, Chile
- 2009-2010 : [Chief of the Laboratory of Environmental Assessment](#) at Linnaeus Laboratories Inc. Role: Environmental impact assessment and monitoring of salmon farms and other productive sectors.
- Mar2008-Feb2009 : Technical Assistant on Environmental impact assessment and monitoring of salmon farms and other productive sectors. Litoral Austral Inc.

Research projects (PI = principal investigator; Co-I = co-investigator)

Period	Role	Duration	Funding agency	Funded amount
2015-2017	Co-I	3 years	Swiss National Science Foundation	USD 200,000
Macroecological and biogeographical patterns in unicellular eukaryotic microorganisms from regional to global scales				
2014	Co-I	1 year	CONACYT Argentina	USD 20,000
Biodiversity and ecology of microbial planktonic frames in austral Patagonia and Antarctic, Argentina (Project PIP N° 418)				
2015	Co-I	1 year	CONACYT Mexico	USD 5,000
Distribution of soil biological crusts along an altitudinal gradient in the Biosphere Reserve Tehuacán-Cuicatlán, México. CONACYT, Mexico				
2014	PI	1 year	Wildlife Conservation Society (WCS)	USD 8,000
Eukaryotic microorganisms inhabiting wetland systems in the Karukinka Park, Tierra del Fuego, Chile				

2008-2009	Co-I	2 years	Universidad de Los Lagos (Chile)	USD 3,000
Foraminifera in salt-marshes from southern Chile: Implications for sea-level studies.				
2008	PI	1 year	FOSIS – Chile	USD 25,000
Survey of flora and fauna in the Llanquihue Lake: Knowledge transfer and management in tourism organizations				
2008	Co-I	1 year	FPA, CONAMA – Chile	USD 6,000
Flora and Fauna of the Guamblin Island National Park, Chile				
2007	Co-I	1 year	IBAM / Forestal Anchile Ltda.	USD 5,000
Environmental quality in lotic and lentic systems in southern Chile				
2007	PI	1 year	Universidad de Los Lagos	USD 3,000
Systematics of freshwater microbial diversity in Los Lagos Region				
2005	PI	1 year	IBAM / Forestal Anchile Ltda.	USD 5,000
Biodiversity in lotic systems associated to monoculture tree plantations				
2002	PI	1 year	Ministerio de Educación (MINEDUC – Chile)	USD 6,000
Creating a scientific collection for the Universidad de Los Lagos				

Science outreach projects (PI = principal investigator; Co-I = co-investigator)

Period	Role	Duration	Funding agency	Funded amount
2006	PI	2 months	Universidad de Los Lagos (Chile)	USD 2,500
Touring exhibition: Microbial life photography exhibition				
2006	Co-I	4 months	Universidad de Los Lagos (Chile)	USD 3,500
A window to the sea: Touring exhibition of marine life				
2006	Co-I	2 months	Universidad de Los Lagos (Chile)	USD 2,000
Compost: A Sustainable Tool - Knowledge transfer to the educational system				
2005	PI	1 year	Ministerio de Educación (MINEDUC – Chile)	USD 6,000
Touring exhibition of regional wildlife – Second Version				
2004	PI	1 year	Ministerio de Educación (MINEDUC – Chile)	USD 6,000
Touring exhibition of regional wildlife – First Version				

Publication record

Papers in journals listed in the SCI

Schiaffino, R.M., Lara, E., **Fernández, L.D.**, Balagué, V., Singer, D., Seppey, C.C.W., Massana, R., Izaguirre, I. (2016) Distribution patterns of planktonic small-eukaryote communities across a Patagonian-Antarctic gradient of lakes. ***Environmental Microbiology***, 18: 5249–5264. [5-Year IF 2014 = 6.0]

Fernández, L.D., Fournier, B., Rivera, R., Lara, E., Mitchell, E.A.D., Hernández, C.E. (2016) Water-energy balance, past ecological perturbations and evolutionary constraints shape the latitudinal diversity gradient of soil testate amoebae in southwestern South America. <i>Global Ecology and Biogeography</i>, 25: 1216–1227. [IF₂₀₁₄ = 6.5] This manuscript was selected to be in the cover of the volume 10, issue 25, of this prestigious journal (check out the cover here: https://goo.gl/Tf8Vrk)
Fournier, B., Coffey, E.E.D., van der Knaap, W.O., Fernández, L.D., Bobrov, A., Mitchell, E.A.D. (2016). A legacy of human-induced ecosystem changes: Spatial processes drive the taxonomic and functional diversities of testate amoebae in <i>Sphagnum</i> peatlands of the Galápagos. <i>Journal of Biogeography</i>, 43: 533–543. [5-Year IF₂₀₁₄ = 4.5]
Delaine, M*, Fernández, L.D*, Armynot du Chatelet, E., Recourt, P., Potdevin, J.-L., Mitchell, E.A.D., Bernard, N. (2016) Cinderella's helping pigeons of the microbial world: the potential of testate amoebae for identifying cryptotephra. <i>European Journal of Protistology</i> 55: 152–164. [5-Year IF₂₀₁₄ = 2.8] *Both authors are co-first authors and contributed equally to the paper.
Fernández, L.D., Lara, E., Mitchell, E.A.D. (2015) Checklist, diversity and distribution of testate amoebae in Chile. <i>European Journal of Protistology</i>, 51: 409–424. [5-Year IF₂₀₁₄ = 2.8]
Fernández, L.D. (2015). Source-sink dynamics shapes the spatial distribution of soil protists in an arid shrubland of northern Chile. <i>Journal of Arid Environments</i>, 113: 121–125. [5-Year IF₂₀₁₄ = 1.6]
Fernández, L.D., Zapata J., Meisterfeld, R., Baessolo L. (2012). First records and community patterns of Arcellinida inhabiting a pristine and remote island from Southeastern Pacific. <i>Acta Protozoologica</i>, 51: 139–154. [5-Year IF₂₀₁₄ = 0.8]
Fernández, L.D., Zapata J. (2010). Distribución de foraminíferos bentónicos (Protozoa: Foraminiferida) en la Ensenada Quillaípe, Chile: Implicaciones para el estudio del nivel del mar. <i>Revista Chilena de Historia Natural</i>, 83: 567–583. [5-Year IF₂₀₁₄ = 0.6]
Fernández, L.D., Zapata J. (2010) Registro tafonómico de <i>Ammonia beccarii</i> (Foraminiferida) en la Ensenada Quillaípe, Chile. <i>Latin American Journal of Aquatic Research</i>, 38: 286–291. [5-Year IF₂₀₁₄ = 0.2]
Fernández, L.D., Rau J., Arriagada, A. (2009) Calidad de la vegetación ribereña del Río Maullín, utilizando el índice QBR. <i>Gayana Botánica</i>, 62: 269–278. [5-Year IF₂₀₁₄ = 0.5]
Zapata J., Fernández, L.D. (2008) Morphology and biometry of <i>Apodera vas</i> [Certes, 1889] from two peatlands in southern Chile. <i>Acta Protozoologica</i>, 47: 389–395. [5-Year IF₂₀₁₄ = 0.8]
<i>Papers in peer-reviewed journals not listed in the SCI</i>
Fernández, L.D., Zamora-Manzur, C. (2015) Dear readers of and contributors to Biodiversity and Natural History. <i>Biodiversity and Natural History</i>, 1: 1 (Editorial).
Fernández, L.D., Zapata J. (2011) Variación estacional en la comunidad de amebas testadas de una turbera temperada del sur de Chile. <i>Boletín de la Sociedad de Biología de Concepción</i>, 80: 27–39.
Fernández, L.D. (2011) Nuevos registros y rangos de extensión ¿Para qué? <i>Boletín de Biodiversidad de Chile</i>, 5: 1–2.
Fernández, L.D. (2010) Foraminíferos del Estuario del Río Contaco, Chile. <i>Boletín de Biodiversidad de Chile</i>, 4: 18–62.

Fernández, L.D. (2010) Extensión del rango de distribución de <i>Saccocirrus sonomacus</i> Martin, 1977 (Polychaeta: Saccocirridae) en el Pacífico Sur. <i>Boletín de Biodiversidad de Chile</i> , 4: 100–102.
Book, book chapters and other publications
Fernández, L.D. (2015) The Quaternary Period. Academy Publish, USA (<i>Book Editor</i>)
Díaz-Aros, R., Fernández, L.D. (2015) The Great American Biotic Interchange: Intercontinental migration of big predators, In: Fernández, L.D. (Ed.), The Quaternary Period. Academy Publish, USA (<i>Book Chapter</i>).
Fernández, L.D. , Briones, E., Martínez, R. (2008) Flora y fauna del Lago Llanquihue, Chile. Centro de Gestión y Desarrollo Empresarial Limitada, Chile. 150 pp. (<i>Technical Report</i>)
Fernández, L.D. , Zapata, J. (2008) Guía para las amebas tecadas de Chile. 150 pp. (<i>Technical Report</i>)
Reports in newspapers/newsletters
2016: “ <i>El cuarteto malvado</i> ” (website of the editorial “Planeta Sustentable”) (In Spanish)
2015: “ <i>La ruta de la Biodiversidad</i> ” (newsletter of the Chilean newspaper ‘La Segunda’) (in Spanish)

Peer-reviewed conference proceedings [I have participated with 40 contributions](#) (posters and/or talks) in different Latin American and European scientific meetings (only a subset of the 5 most recent contributions are shown)

Fernández L.D. , Lara, E., Seppey, C.V., Singer, D., Fournier, B., Mitchell, E.A.D., Bernard, N. (2016) The patterns and causes of latitudinal and altitudinal diversity patterns in free-living soil unicellular eukaryotes: Using soil testate amoebae as model organisms. In: 8th Meeting of the International Society for Testate Amoebae, 12-14 September, Ilhabela, Brazil. <u>Oral presentation.</u> This presentation was awarded with the YoTAR award (Young Testate Amoebae Researcher award).
Fernández L.D. , Rivera, R., Fournier, B., Mitchell, E.A.D., Lara, E., Hernández, C.E. (2015). Water-energy balance, past ecological perturbations and evolutionary constraints shape the latitudinal diversity gradient of soil testate amoebae in southwestern South America. In: VII ECOP - ISOP joint meeting. 5-10 September, Seville, Spain. <u>Oral presentation.</u>
Fernández, L.D. , Lara E., Schiaffino R., Izaguirre I. (2015). A lake-dwelling microalga exhibits population genetic structure among lacustrine populations in southern Argentinean Patagonia. In: 34th Annual Meeting of the German Society for Protozoology, 3-6 March, Magdeburg, Germany. <u>Oral presentation.</u>
Fernández L.D. , Rivera, R., Fournier, B., Mitchell, E.A.D., Lara, E., Hernández, C.E. (2015). Soil testate amoeba diversity follows a unimodal latitudinal pattern in southwestern South America caused by short- and long-term processes. In: 34th Annual Meeting of the German Society for Protozoology, 3-6 March, Magdeburg, Germany. <u>Oral presentation.</u>
Fernández L.D. , Rivera, R., Fournier, B., Mitchell, E.A.D., Lara, E., Hernández, C.E. (2015). Soil testate amoeba diversity follows a unimodal latitudinal pattern in southwestern South America caused by short- and long-term processes. In: 7th Meeting of the International Society for Testate Amoebae, 8-12 September, Poznan, Poland. <u>Oral presentation.</u>

Other scientific activities

Founder and Editor-in-Chief of the scientific journal *Biodiversity and Natural History* (formerly Boletín de Biodiversidad de Chile) (<http://www.biodiversnathist.com/>) (since 2009 to present)

I have been asked to review manuscripts on the following scientific journals: Boletín de Biodiversidad de Chile, Biodiversity and Natural History, Journal of Bacteriology Research, Journal of Eukaryotic Microbiology, European Journal of Protistology, Journal of Limnology, Hydrobiologia, Protist, Microbial Ecology.